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Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

United States. NATIONAL INSTITUTE OF MENTAL HEALTH.

October 1, 1979 - September 30, 1980

VOLUME II

INDIVIDUAL PROJECT REPORTS

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Division of Clinical and Behavioral Research,
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NATIONAL INSTITUTE OF MENTAL HEALTH

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PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Central Amines and Psychiatric Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A collaboration between the National Institute of Mental Health (NIMH) and the National Naval Medical Center (NNMC) involves the study of central amine metabolites in the cerebrospinal fluid (CSF) of psychiatric patients, largely personality disorders and alcoholics. Results to date indicate that aggressive behavior may be inversely correlated with CSF 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with CSF 3-methoxy-4-hydroxyphenyl glycol (MHPG) and cyclic 3',5'-adenosine monophosphate (c-AMP). Alcoholics appear to have an increased 5HIAA in relation to acute intoxication. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE); low CSF dopamine-β-hydroxylase (DBH), low platelet monoamine oxidase (MAO), low plasma amine oxidase (AO) and high red-cell catechol-O-methyl transferase (COMT) are related to adverse reactions to disulfiram. CSF DBH also appears to be related to certain personality measures in the MMPI. Within these populations neither clinical depression nor aggressive behavior appears to be related to alcoholism. These patients have also been used as control data for other psychiatric diagnostic groups at NIH.

Project Description:

Objectives: Evidence obtained in recent years indicate that epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5HT), and gamma-amino butyric acid (GABA), among others, act as central nervous system neurotransmitters and/or modulators of central nervous system (CNS) transmission. Although considerable indirect pharmacologic evidence has linked these amine systems with psychiatric illnesses (particularly affective illness and schizophrenia), the relative lack of direct data in man has limited the applicability of these hypotheses to improved diagnosis and treatment of the major psychiatric disorders. Relatively little direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals Recent data suggests that affective disorders and psychoses may yield to biological sub-typing which would enable a more effective choice of pharmacological therapy for individual patients. There has been virtually no data on central neurochemical function in the various personality disorders--a rather striking deficit in our knowledge, considering the evidence suggesting that some personality disorders have patterns of family history suggestive of a genetic component. Data from animals suggests a relationship between aggressive behavior and biogenic amines. Neurotransmission in alcoholism also needs more study. The purpose of this present project would be to extend the studies of central amine turnover into larger populations of psychiatric patients.

Methods Employed: A technique has been developed at NIMH which allows for the estimation of brain amine turnover in psychiatric patients. This method involves the administration of probenecid, a drug which blocks the process by which the amine metabolites are removed from the brain-cerebrospinal fluid (CSF) system into the blood. Following blockade of their removal, the amine metabolites accumulate in the CSF at a rate which is in proportion to their synthesis or turnover rate in the brain. Experiments with this technique at the NIH have indicated the following: 1) experience in many patients has shown that both the lumbar puncture and the administration of probenecid are safe procedures not associated with significant side effects or toxicity, except for nausea; 2) studies of this technique in conjunction with specific amine precursors and synthesis inhibitors have provided evidence that the probenecid induced accumulation of amine metabolites does reflect amine synthesis and turnover rates; 3) application of this technique has shown differences in central amine function among certain subgroups of psychiatric patients.

Major Findings: About 175 patients have participated in this study to date. Preliminary findings suggest that a group of personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment, when compared to a mixed control group from NIMH (normals and patients with non-CNS neurological disorders), have similar baseline 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG). Furthermore, results to date indicate that aggressive behavior may be inversely correlated with

5HIAA and positively correlated with MHPG. A group of personality disorders have also shown no statistically significant difference in CSF cyclic 3',5'-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from groups of depressive, manic, and schizophrenic patients, though aggressive behavior is positively correlated with c-AMP. Those patients who were administratively discharged from the service and those with prior history of suicidal attempts have shown lower CSF 5HIAA and higher MHPG, c-AMP and c-GMP.

A group of alcoholics have also been studied. It has been found that alcoholics do not differ from personality disorders with respect to HVA. However, studies do indicate that the mean 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in relation to the length of abstinence to reach a baseline value not differing from the personality disorders. An additional study shows that, though HVA values do not change in relationship to the time period post intoxication-withdrawal, they are depressed concomitant with the use of disulfiram (Antabuse), a dopamine-β-hydroxylase (DBH) inhibitor. In addition, disulfiram use correlates with a significant increase in serum NE. Mean serum DBH in alcoholics versus normal controls was significantly lower, blood pressure was significantly higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Other studies indicate that lower DBH is correlated with increasing psychopathology as measured by the Minnesota Multiphasic Personality Inventory (MMPI) and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-O-methyl transferase (COMT) are associated with disulfiram-induced psychoses.

Other studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism. Aggressive behavior can be associated with alcoholism when the alcoholics are also personality disorders.

Significance for Mental Health Research: CNS functioning is greatly under-studied in two major groups of psychiatric patients, namely personality disorders and alcoholics. Studies of animal models and hyperactive children suggest a relationship between central neurotransmitter systems and aggressive behavior. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also drug-free personality disorders may serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. This progress of the project is submitted to the Navy for reapproval each March. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC.

Publications:

1) Smith, C.C., Tallman, J.F., Post, R.M., van Kammen, D.P., Jimerson, D.C., Brown, G.L., Brooks, B.R., Bunney, W.E., Jr: An examination of baseline and drug-induced levels of cyclic nucleotides in the cerebrospinal fluid of control and psychiatric patients. Life Sci 19:131-136, 1976.

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3) Major, L.F., Ballenger, J.C., Goodwin, F.K., Brown, G.L.: Cerebrospinal fluid homovanillic acid in male alcoholics: effects on disulfiram. Biol Psychiatry 12(5):635-642, 1977.

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6) Brown, G.L., Goodwin, F.K., Ballenger, J.C., Goyer, P.F., Major, L.F.: Aggression in humans correlates with cerebrospinal fluid amine metabolites, Psychiatry Research 1:131-139, 1979.

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8) Brown, G.L., Goodwin, F.K., Ballenger, J.C., Goyer, P.F.: Cerebrospinal fluid amine metabolites and cyclic nucleotides in human aggression. Psychopharmacol Bull, in press, 1980.

9) Jimerson, D.C., Post, R.M., van Kammen, D.P., Skyler, J.S., Brown, G.L., Bunney, W.E. Jr.: CSF cortisal in depression and schizophrenia. Am. J. Psychiatry, in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00021-15 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of Sleep

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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- See Attached Continuation -

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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This unit, which overlaps both the Biological Psychiatry Branch and the Laboratory of Clinical Psychopharmacology, has conducted studies on both clinical and basic aspects of sleep for many years. This unit has made major attempts both to characterize the sleep disorder of depression, and to understand its mechanism, both from the point of view of neuropharmacological models and underlying circadian rhythms. Our findings suggest that muscarinic supersensitivity may be present in patients with primary affective illness both in remission and when ill. Our data suggest that patients with primary affective illness demonstrate muscarinic supersensitivity as measured by pupillary constriction to pilocarpine and by the Cholinergic REM Induction Test.

The unit has also made major investigations on neuroendocrine relationships to sleep-wakefulness and to certain specific sleep disorders, including insomnia.

Our studies on human learning and memory suggest that cholinergic agonists facilitate specific types of cognition, whereas blockade of catecholamine neurotransmission inhibits cognitive performance.

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Department of Medicine, University of Rochester, Rochester, New York

Project Description:

Objectives:

Experimental findings over the past decade promise understanding of the basic biochemical processes underlying sleep; and increasingly clear demonstrations of the severity of sleep disturbances in many psychiatric patients indicate the need for better means of alleviation. To whatever extent understanding of sleep can be achieved, more effective treatment of its disturbances will surely follow.

I. Major Findings: Pharmacological Studies of Sleep

A. Cholinergic

1. Time-dependent Effects of Physostigmine on Normal Human Sleep and Arousal

Physostigmine induces REM sleep or arousal in man depending upon the dose and time of administration. Thirty-three normal volunteers received 40 physostigmine (0.5 mg over 2-3 min) and 40 matched saline (sal) infusions. (1) With postinfusion-arousals excluded, infusions given 5 min after sleep onset reduced time from infusion to onset of REM from 78.9 ± 14 min (sal) to 52.4 ± 10.4 (phy) ($p<0.05$, two-tailed "t" test). (2) Infusions given 35 min after sleep onset reduced time from infusion to onset of REM from 65.7 ± 15.2 (sal) to 11.2 ± 2.1 (phy) ($p<0.005$). REM was induced more rapidly by physostigmine infusions given 35 min after than 5 min after sleep onset ($p<0.01$). (3) For the infusions at REM onset, 5 min after and 25 min after REM, the predominant effect was one of arousal. Judging from number and duration of awakenings, the order of intensity of arousal was: REM > 5 min after > 25 min after REM. Although a dose of 0.5 mg awoke the subjects when given 25 minutes after the first REM period, a dose of 0.25 mg induced REM.

These data suggest that cholinergic mechanisms are involved in initiating REM sleep and arousal. During the first NREM period, physostigmine more readily induces REM when given 35 min than when given 5 min after sleep onset, whereas the same dose given at REM onset and during the second NREM period produces different intensities of arousal.

In order to determine whether the duration of REM could be affected, seven normal male volunteers (mean age 22.8) were studied for three nights (one "adjustment" and two experimental nights). Subjects were pretreated with 0.75 mg IM methscopolamine prior to bedtime; and during sleep they received either placebo or physostigmine 1.0 mg given by a slow IV drip over a period of 60 minutes. The IV infusion began 35 minutes after sleep onset and ended about 95 minutes after sleep onset. Physostigmine significantly decreased both the latency of the first REM period (Placebo = 108.4 ± 16.2 min (mean ± S.E.) physo = 61.7 ± 2.2 min, $p<.05$ and the duration of the second NREM period (placebo 110.4 ± 13.6, physo = 74.4 ± 6.5, $p<.05$). The duration of individual REM periods was n altered. Although total REM time and REM percent showed a non-significan increase of physostigmine (RT = 98.2 ± 6.4 min, R% = 25 ± 1.5%) as compar to placebo nights (RT = 80.9 ± 9.4, R% = 20.1 ± 1.9%) this can be account for by the greater number of REM periods on physostigmine (4.3 ± 0.3) tha on placebo (3.6 ± 0.2) conditions. Cholinergic mechanisms may modulate t "timing" rather than the "maintenance" of REM.

2. Physostigmine-induced REM is Associated with Normal Dreaming

To examine the psychological process of dreaming in man, seventeen normal volunteers (11M, 6F, mean age 23), after pretreatment with methscopolamine 0.5 mg IM, slept with an IV needle, and at either 10 or 35 minutes after sleep onset received one intravenous infusion per night of either placebo or physostigmine 0.5 mg.

During Experiment I subjects were given an IV infusion 10 min after sleep onset and were awakened 20 min after infusion. Experiment II included awakening either 7 or 20 min following an infusion given 35 min after sleep onset; in addition subjects were instructed to go back to sleep after the 20 min awakening and were then awakened approximately 7 min after they had spontaneously gone into REM sleep. The above study was designed to control for (1) time of infusion, (2) time from infusion to awakening, (3) state of sleep prior to awakening.

At each awakening a taped interview was conducted. Subjects were questioned waking. After the interview subjects filled out a questionnaire. A dream mentation scale was constructed from the questionnaire ranging from +5 (absolute confidence in presence of dream) to -5 (absence of dreaming).

Comparison of Dream Reports from Physostigmine-induced and Spontaneous First REM Period

		Physostigmine-induced REM Periods	Spontaneous REM Periods	p
1)	No. of REM awakenings	9	10	NS
2)	No. of dream reports*	8	7	NS
3)	Duration of REM prior to waking (mean±SEM min)	6.6 ± 0.6	7.1 ± 0.4	NS
4)	REM Density (0-8 scale)	1.7 ± 0.2	1.7 ± 0.4	NS
5)	Vividness of dreams*	3.1 ± 0.4	2.7 ± 0.4	NS
6)	Unusualness (0-5)*	1.6 ± 0.5	1.7 ± 0.3	NS
7)	Emotionality (0-5)*	2 ± 0.4	1.9 ± 0.5	NS

* These scores were obtained from a questionnaire filled out independently by both subjects and experimenter and from a structured interview conducted by the experimenter.

During Experiment I and the 7 minute post-infusion awakenings of Experiment II, neither REM sleep nor dreaming was induced by physostigmine. In the 20 minute post-infusion, physostigmine induced the onset of REM sleep in approximately 13 minutes in all but one subject. Those drug-induced REM episodes were associated with a significantly higher incidence of dream recall than placebo.

This study indicates that the physostigmine-induced physiological characteristics of REM sleep are accompanied by dreaming. The dreaming occurring during physostigmine-induced REM periods appears to be similar to that observed during spontaneously occurring REM sleep.

3. Induction of REM Sleep in Man by Arecoline, a Cholinergic Muscarinic Agonist: Blockade by Scopolamine

Ten normal volunteers (8M, 2F, mean age 25.3) were each studied on 4 randomly ordered experimental nights (after a night of adaptation). After pretreatment with methscopolamine 0.5 IM on three of the experimental nights, they each received one intravenous infusion about 32 to 35 min after sleep onset of either (1) placebo, (2) arecoline 1.0 mg IV, or (3) arecoline 1.5 mg IV. On the fourth night pretreatment was scopolamine 0.5 mg IM followed by IV infusion of arecoline 1.5 mg. The results were: (1) Both arecoline 1.0 mg and 1.5 mg triggered the onset of REM sleep, 20.1 and 15.9 min after infusion, respectively. (2) Pretreatment with scopolamine blocked the REM-inducing effect of arecoline 1.5 mg IV. (3) Once the onset of the first REM period was moved forward, subsequent nonREM-REM cycles were advanced without altering REM-REM intervals. (4) The number of REM periods was significantly increased after arecoline 1.0 mg (4.1±0.3) and 1.5 mg (4.5±0.2) compared to placebo (3.5±0.2). (5) Total REM time and percent after arecoline 1.5 mg (100.7±6.4 min, 24.7± 1.7%) was significantly greater than placebo (76.2±5.4 min, 18.1±0.9%). The data with arecoline, a specific cholinergic muscarinic agonist, confirms our previous findings with physostigmine and suggests that cholinergic stimulation of muscarinic receptors may play a role in the triggering of REM sleep in man. In addition, the results suggest that both arecoline and physostigmine "phase advance" REM sleep towards the onset of sleep; that is, all REM periods occur earlier in the night than under normal conditions.

4. Experimental Shortening of REM Sleep Ultradian Rhythm with Timed Multiple Infusions of Arecoline

Seven normal volunteers (4M, 3F, mean age 25 yrs) were studied under the following two experimental conditions. (1) Pretreatment with methscopolamine 0.5 mg IM followed by three IV infusions of arecoline (1.5 mg, 1.0 mg and 1.0 mg doses) given at the following three times respectively: (a) 35 min after sleep onset; (b) 30 min after end of first REM period; (c) 30 min after end of second REM period.

(2) Pretreatment with methscopolamine followed by three placebo (saline) IV infusions. Subjects were run either in pairs or groups of 3 such that the timing of the placebo infusions was "yoked" to the arecoline infusions.

TABLE: Duration (Minutes) of NREM and REM Periods (Mean ± SEM)

	$NREM_1$	REM_1	$NREM_2$	REM_2	$NREM_3$	REM_3	$NREM_4$	REM_4	$NREM_5$	REM_5
Placebo	95.7	14.1	89.1	22.8	78.4	25.5	-	-	-	-
	±14.7	±3.9	±5.7	±3.2	±4.6	±4.7	-	-	-	-
Are-	42.2*	10.3	38.3*	12.8*	41.4*	21.0	84.7	22.1	65.4	28.8
coline	±2.6	±2.4	±1.3	±2.5	±3.7	±4.9	±4.1	±5.4	±4.6	±5.0
								(N=6)	(N=6)	

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*p<.01

The results of this study are: (1) It was possible to transform a normal REM ultradian rhythm (with REM-REM interval of about 100 min) into a rhythm with REM_1-REM_2 interval of 48.6 min and REM_2-REM_3 interval of 54.2 min. (2) There were a significantly greater number of REM periods after arecoline (5±0.22) than placebo (3.71±0.29) resulting in greater REM time and percent (arecoline = 104.1±1.5.6 min, 24.8±1.9% and placebo = 84.4±4.6 min, 20.1±1.2%). (3) Duration of individual REM periods was either unchanged or shortened (e.g., REM_2). Acetylcholine plays a role in the timing of REM sleep. The increase in REM time seen appears to result from an increase in the number but not the duration of REM periods. There also appears to be a "ceiling" after which REM time cannot be further increased by cholinergic agents since both single and multiple infusions of arecoline produce the same amount of increase in REM time.

5. Acetylcholine and Sleep: Effect of Oral Choline on Normal Human Sleep

Choline, a normal dietary constituent, has recently been shown to be a precursor of brain acetylcholine. If so, in the light of our previous work with physostigmine and arecoline we would predict that choline administered to humans may alter REM sleep parameters. A double-blind crossover design with 12 normal male subjects was used. Subjects slept for 3 nights (1 adaptation and 2 experimental) and just prior to "lights out" (11:30 p.m.) were given an elixir containing either 10 g of choline chloride or placebo matched for taste, color and consistency.

Effects of Choline Chloride (10 gm) or Placebo
Upon Sleep of Normal Volunteers (N=12)

	Placebo	Choline (10 gm)
Total Recording Period	472 ± 5*	492 ± 4
Sleep Latency	33 ± 8	24 ± 4
Total Sleep	422 ± 8	446 ± 6
Non-REM	326 ± 10	347 ± 8
Stage I	12 ± 3	10 ± 3
Stage II	269 ± 7	273 ± 9
Stage III	22 ± 4	29 ± 4
Stage IV	25 ± 5	33 ± 5
Stage V	25 ± 5	33 ± 5
REM Sleep	95 ± 6	100 ± 5
REM Latency	92 ± 12	107 ± 12
Intermittent Awake	13 ± 4	17 ± 5
Early Morning Awake	4 ± 1	3 ± 1

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* mean ± SEM
values are in minutes

The results were: (1) Choline did not have any effect on conventional sleep parameters when data from all 12 subjects were analyzed. (2) When the subjects were rank ordered with respect to their placebo (baseline) REM latency, REM time and REM %, we found that low placebo REM latencies were increased and high placebo REM latencies were decreased by choline. The correlation between placebo (baseline) REM latency and the change in REM latency after choline (i.e., choline-placebo value) was inversely correlated $r=-0.90$, $N=12$ ($p<.01$). Similarly there was an inverse correlation between placebo REM time and REM % and change after choline ($r=0.63$ for REM time and $r=0.61$ for REM%, both $p<.05$). Whether the above changes in REM parameters produced by choline represents a genuine central neuromodulatory effect or is no more than a statistical artifact is unclear. Likewise neither choline (7.5 gm) at 8 am or 11 pm nor choline (7.5 gm) at both 8 am and 11 pm had any affect on sleep as compared with control. These results suggest choline may not enhance functional cholinergic neurotransmission, at least not in putative sleep centers.

6. The Effect of Piperidine, a Cholinergic Nicotinic Agent on Normal Human Sleep

Piperidine is an alicyclic amine with nicotine-like cholinomimetic effects on central and peripheral nervous system. In view of conflicting reports on the effect of piperidine on REM Sleep in rodents, we undertook to study the effect of oral piperidine HCl in normal volunteers. Using a 4x4 Latin square design, 8 subjects were recorded on 4 nights under the following four experimental conditions (a) placebo, (b) piperidine 200 mg, (c) piperidine 400 mg and (d) piperidine 600 mg.

All medications were given prior to "lights out" (11:30 p.m.). No change in any sleep parameter including REM latency, REM time and REM density, was observed after piperidine. Although data from animal pharmacology suggests that the doses used in our study would have central effects, the possibility still remains that physiologically effective doses were not used. Intravenous administration of piperidine during sleep is also currently underway.

7. Scopolamine-induced Muscarinic Supersensitivity in Normal Man: Changes in Sleep

An injection of scopolamine (6μg/kg) was administered on 3 consecutive mornings to normal human subjects. Sleep recordings obtained at night (when the central anticholinergic effect of the morning scopolamine was no longer present) indicated a significant reduction in latency to REM sleep onset on the nights following the second and third injection. REM latency was significantly reduced on the second (50 min ± 12) and third (45 min ± 13) scopolamine nights as compared with baseline (96 min ± 16) ($p<.005$). This effect is opposite to the direct pharmacological action of nighttime administration of scopolamine (i.e., prolongation of REM latency). Furthermore, scopolamine pretreatment on 2 consecutive mornings also potentiated the REM-inducing effect of arecoline, a central muscarinic agonist. The time from infusion to REM was 17 min ± 9 after scopolamine pretreatment and 47 min ± 11 after saline ($p<.05$). These data are consistent with development of cholinergic supersensitivity following cholinergic blockade.

8. Identification of Stimulatory Cholinergic Mechanisms in Sleep-related Growth Hormone Secretion

We have examined the effects of cholinergic blockade with methscopolamine bromide, 0.5 mg intramuscularly, on sleep-related and insulin-induced growth hormone (GH) and prolactin (PRL) secretion. Thirteen normal young men were studied; eight had sleep studies, and at different times in 3 sleep subjects and in 5 others not studied during sleep, insulin tolerance tests were performed with 0.1 unit/kg insulin. After an adjustment night in the sleep laboratory, saline control and methscopolamine studies were done in a random sequence; study procedures included electroencephalographic, electromyographic, and electro-oculographic recordings, and blood sampling every 20 minutes for hormone radio-immunoassays. On methscopolamine nights, the mean ± SEM overall GH level of 2.89 ± 0.44 and the mean peak GH level of 11.09 ± 3.11 ng/ml were dramatically reduced to 0.75 ± 0.04 and 1.04 ± 0.25 ng/ml, respectively ($p<0.0001$ and $p<0.001$). Despite virtual absence of GH secretion during the night in every study subject, no measured sleep characteristic was significantly affected by methscopolamine, including percentage slow-wave sleep (12.1 ± 2.6% control vs. 10.3 ± 2.5% drug). Sleep PRL concentrations were not changed by methscopolamine. In contrast to the abolition of sleep-related GH secretion, administration of methscopolamine only marginally reduced the GH response to insulin hypoglycemia. Only one of ten time points differed significantly, and the peak concentrations, mean increments, and areas under the curves were not significantly different. We conclude that the burst of GH secretion which normally occurs following sleep onset is primed by a cholinergic mechanism which does not influence slow-wave sleep. Cholinergic mechanisms outside the central nervous system do not appear to play an important role in sleep-related prolactin secretion. The neurotransmitter mechanisms, and presumably the pathways, which subserve sleep-related GH secretion in man appear to be different from those which mediate the GH response to pharmacologic stimuli such as insulin.

9. Piperidine Enhances Sleep-related and Insulin-related Growth Hormone Secretion

Seven male normal adult volunteers slept in the laboratory, and blood samples were drawn every 20 minutes, after the lights were turned out at 11:00 p.m. A 30 minute IV infusion of piperidine or saline was started at sleep onset. In a comparison study, eight other volunteers had blood samples drawn for 2 hours after receiving 0.1 unit/kg regular insulin at 8:00 a.m. A 30 minute infusion of 100 mg piperidine or saline was started 15 minutes before insulin stimulation.

An analysis of variance revealed that piperidine significantly ($p<0.01$) enhanced sleep-related GH secretion over the 8 hours, from 4.22±0.46 ng/ml on saline to 6.09±0.78 ng/ml on piperidine ($p<0.02$ by paired t-test). This was particularly striking in the first two hours, when the values rose from 7.18±1.23 to 15.16±2.88 ng/ml ($p<0.02$). Similarly, there was enhancement of GH secretion during daytime insulin testing. The maximum GH increase after saline was 36.8±3.6 ng/ml compared to 48.0±4.3 ng/ml after piperidine ($p<0.01$). In order to determine whether the enhanced GH secretion was simply a summation of responses to insulin and piperidine separately, we also infused piperidine 100 mg alone in seven volunteers. There was no effect on GH concentrations. Prolactin concentrations were also measured during the sleep and insulin studies; there was no significant effect of piperidine.

In sum, this provides further evidence of cholinergic mechanisms in the regulation of these forms of GH secretion, although the relative roles of nicotinic and muscarinic receptors are still unclear. There is little evidence that the cholinergic system plays a significant role in sleep-related or insulin-induced prolactin secretion.

10. Choline Chloride Reduces Insulin-induced Growth Hormone Secretion without Affecting Sleep-related Growth Hormone

Choline chloride (10 gm) or placebo were administered one hour prior to testing the effect of insulin-induced growth hormone release or prior to bedtime. Choline significantly reduced the insulin-induced growth hormone peak from 24 ± 4 to 17 ± 4 ng/ml ($p<.01$). No effect on nocturnal growth hormone or prolactin release was noted. The mechanism by which choline affected insulin-induced growth hormone is not clear, but probably does not result from increased cerebral acetylcholine synthesis.

B. Histamine: Effects of L-Histidine on Sleep

There is considerable indirect evidence suggesting that histamine may function in the brain as a neurohumor in sleep-waking mechanisms. To test the possible role of histamine in the control of waking in man, we administered its amino acid precursor, L-histidine, to human subjects and evaluated their electroencephalographic sleep patterns. L-histidine was given to three patients with intractable narcolepsy (20 gm per day for 2 weeks), to four normal volunteers (32.4 gm per day for 5 days) and to a patient with progressive systemic sclerosis (48.6 gm per day for 16 days. No effects were observed on nocturnal EEG sleep patterns in any of the subjects or on the symptoms of the narcoleptic patients. Although the degree to which histamine levels in the brain were elevated in this study is not clear, these results do not encourage the hypothesis that histamine is an alerting or waking factor in the brain.

C. Catecholamines

1. Differential Effects of D- and L-Amphetamine on the Sleep of Depressed Patients On and Off Lithium Treatment

Dextro (d-) and levo (l-) amphetamine produced different EEG sleep changes in seven depressed patients. Each patient received placebo or one of the isomers (30 mg base) at 7:25 a.m. in a double-blind fashion. The patients were studied under two conditions: without treatment with lithium carbonate and with treatment with lithium carbonate (0.9-2.1 gm/day beginning a minimum of 10 days before study). Both isomers reduced REM sleep and the proportion of total sleep spent in REM (the REM%). No REM rebound was observed on the night following REM suppression. Only d-amphetamine delayed sleep onset and reduced total sleep time, NREM sleep time, and sleep efficiency. The same changes were observed with and without lithiu carbonate treatment.

2. Pimozide Attenuates d-Amphetamine-induced Sleep Changes in Man

Pretreatment with pimozide (mean dose=13 mg/day) blocked the effect of d-amphetamine (20 mg base, administered by intravenous bolus infusion at 0815) on all-night EEG sleep patterns in seven hospitalized psychiatric patients. Each patient was studied for five nights (2 nights baseline, 1 night on the day of the infusion, and 2 nights recovery) with and without pretreatment with pimozide. Without treatment with pimozide, d-amphetamine significantly reduced duration of total sleep, REM and nonREM sleep, Stage I, and Stage II. With coadministration of pimozide, d-amphetamine has no effect on sleep. These results suggest that the d-amphetamine-induced changes in sleep are mediated by dopaminergic neurons.

D. Serotonin:

1. Relationship to Narcolepsy

Since serotonin has been implicated in the induction and maintenance of sleep, we administered para-chlorophenylalanine (PCPA 3 gm/day for up to 4 weeks) to 3 patients with narcolepsy. No consistent change in sleep or clinical symptomatology was observed in the 3 patients as a group. One patient, in contrast to our previous experience with PCPA in patients with carcinoid syndrome, actually showed a significant increase in REM while on PCPA, while the other patients showed no decrease in REM. These results suggest (a) that narcolepsy is not related to an abnormality of serotonin and (b) that narcoleptics respond differently to PCPA than carcinoid patients.

2. Reduction of Brain Serotonin and REM Sleep by Acute Administration of a Tryptophan-free Amino Acid Diet

A variety of studies have suggested that the serotonergic system plays an important role in the regulation of sleep. In order to clarify this role, we have performed sleep studies on rats in whom brain serotonin was decreased by a new technique--the acute administration of a tryptophan--free amino acid diet.

Sleep studies were performed on five rats in a paired-data, crossover design. As can be seen on the Table, percentage REM sleep was decreased by one-third ($p<.05$), and there was a small but significant increase in nonREM sleep. Total sleep time was unchanged.

Our observation that an acute reduction in brain serotonin failed to produce decreases in nonREM sleep is in agreement with the results of two studies each in rats and humans. These data seem inconsistent with the hypothesis that the serotonergic system plays an important role in the production of nonREM sleep.

	Control Diet	Tryptophan-Free Diet	Statistical Significance
Total sleep	519.6 ± 25.7*	529.8 ± 27.0	NS
Sleep latency	45.5 ± 10.6*	35.8 ± 10.6	NS
Intermittent waking (IW/TRP)	33.9 ± 3.0	30.8 ± 2.2	NS
NonREM sleep (NR/TST)	87.6 ± 2.4	91.4 ± 2.3	$p<0.05$
REM sleep (REM/TST)	12.3 ± 2.4	8.5 ± 2.2	$p<0.05$

Values represent mean number of 30 sec. epochs ± SEM.

Effects of tryptophan-free diet on brain serotonin and 5HIAA

Hours after food presentation	Control diet Serotonin	Control diet 5HIAA	Tryptophan-free diet Serotonin	Tryptophan-free diet 5HIAA
2	1.43±0.04	0.52±0.06	0.80±0.02***	0.38±0.02**
4			0.62±0.01***	0.27±0.02***
8			0.44±0.02***	0.21±0.01***
24	1.52±0.02	0.56±0.04	0.65±0.02***	0.41±0.04*

Each value represents the mean from four rats ± S.E.M., in units of μg/g brain tissue.

* $p<0.05$ by t-test when compared to mean of combined control values.
** $p<0.01$
*** $p<0.001$

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of clinical disorders, biochemistry and sleep.

3. Intravenous Chlorimipramine Affects REM Cycle in Patients with Excessive Daytime Sleepiness and Control Subjects

The sleep of 5 normal volunteers and 4 patients with excessive daytime sleepiness was studied following intravenous administration of 5 mg chlorimipramine during the first period of rapid eye movement (REM) sleep. There was a 95% increase in the duration of the first REM cycle (time lapsed from onset of first REM period to the onset of the second REM period) in all 9 subjects, $p<.01$, compared to saline control nights. Other effects included a significant, $p<.05$, mean decrease in the post-infusion REM time and REM percentage to approximately one half of the control levels. Phasic events associated with REM sleep were also diminished during the post-infusion period with significant mean decreases in the REM density and the REM index per minute of sleep.

E. Ethanol:

1. A Dose-response Study of the Acute Effects of Ethanol on the Sleep of Rats

We have administered increasing doses of ethanol (1.1, 1.5, 2.0, 2.5 g/kg) and equivolume saline intraperitoneally to five groups of six rats each. Injections were given five minutes before the beginning of seven-hour recordings, from 9:00 a.m. to 4:00 p.m. The percent of REM decreased and the percent of slow-wave sleep increased. This effect occurred most strongly in the first 3.5 hours of sleep. The number of awakenings were significantly increased in the second half of the recording. This may be a reflection of greatly decreased blood alcohol levels. (A dose of 2.5 g/kg is completely metabolized in about six hours in the rat.) It is of interest that even the highest doses of ethanol did not produce prolongation of total sleep time in either the whole recording or the first or second halves.

2. Hypnotics and Ethanol: Residual EEG Effects

The literature is divided between those who have found a possible synergistic interaction of ethanol and benzodiazepines and those who have not. We have examined this relationship by recording 12-minute waking clinical EEG's in 47 normal young men who received 0.25 mg triazolam, 30 mg flurazepam or placebo, alone or in combination with 0.8 g/kg ethanol. EEG's were performed as a baseline (3:00 p.m.), 50 minutes after receiving drug or drug placebo (5:50 p.m.), 50 minutes after receiving ethanol or ethanol placebo (7:05 p.m.) and 16 hours after having received the drug (9:00 a.m.). Criteria for the presence of drug effects were:

1. Increase in amount and amplitude of sinusoidal rhythmic beta activity.
2. Lower amplitude of background activity.
3. Decrease in amount of alpha activity.
4. Presence of "well-modulated" alpha.
5. Presence of "drug spindles."

Eighty-five percent of the baseline records were correctly identified as having no drug effect. Analysis of the second EEG (50 minutes after drug) and third EEG (50 minutes after ethanol) showed that our interpretations had a significant relationship to whether or not the subject had received the active drug ($p<0.02$ and $p<0.05$, respectively). These findings lead us to believe that this method for determining the presence of drug effects is valid.

Analysis of the fourth EEG--16 hours after drug administration--showed no overall difference in incidence of EEG drug effects among the various combinations of drugs and ethanol. Neither drug alone was different from placebo, nor was either drug alone different from itself plus ethanol.

Flurazepam plus alcohol produced drug effects in 86% of the records, compared to 22% in the placebo group ($p<0.019$; Fisher Exact Probability Test). The difference between incidence of drug effects following triazolam plus ethanol (56%) and placebo (22%) did not reach statistical significance. There was no difference in frequency of effects between flurazepam plus ethanol and triazolam plus ethanol.

These data lead us to believe that ethanol produces a small but significant increase in the duration of EEG effects of flurazepam. Flurazepam alone is known to remain active for some time (the major metabolite may have a half-life of 50-100 hours). Thus, repeated use of the drug might produce cumulative effects which may possibly be increased by ethanol.

3. Sleep, Behavior, and Blood Ethanol Concentration in the Ethanol-Dependent Rat

In this study sleep recordings were performed to further characterize a previously-described animal model of ethanol dependence. In summary, this consists of administering approximately 10 gm/kg of ethanol per day by intragastric intubation in six divided doses for four days. Eight-hour EEG recordings were made during the baseline period and the first and third days of ethanol administration (induction period). One group of animals (N=14) was also recorded on the first and fourth withdrawal days; a second group (N=5) was recorded on days 1-4 and 6 of withdrawal. During the induction period, REM sleep time was decreased, particularly on the third ethanol day when blood ethanol concentrations were highest.

This largely reflected decreases in the number of REM sleep episodes. The first day of ethanol withdrawal was associated with a decrease in total sleep time, primarily due to loss of nonREM sleep. Total sleep time gradually returned to normal, and in fact slightly exceeded baseline levels on the third withdrawal day. At this time, REM percentage increased to twice the baseline values. This was related to a decrease in REM latency and an increase in the number of REM sleep episodes. REM percentage then decreased such that on the fourth withdrawal day it approached baseline values. Total sleep time and other sleep parameters were also indistinguishable from baseline on the fourth day. Blood ethanol concentrations on the first withdrawal day were inversely related to behavioral scores of withdrawal and to EEG sleep latency.

The major observations in this model seem consistent with observations in the literature on ethanol withdrawal in animals and humans. Several of th EEG parameters--notably the initial decreased sleep and later REM rebound— may be useful in testing new treatments for ethanol dependence.

F. Enzymes: The Relation of Platelet MAO to Human Sleep

We have examined the sleep of normal volunteers with high and low platelet MAO activity. These volunteers were drawn from groups with MAO activity in the highest and lowest decile of a population of 375 students. Ten high {mean 16.1±3.2 (sd) nanomoles per 10^8 platelets per hour} and ten low {7.1±2.3 (sd)} volunteers, 5 men and 5 women in each group returned fo sleep studies. Each subject was recorded for two nights following two adjustment nights.

The group with high platelet MAO activity had significantly lower REM slee time (69.9±5.2 vs 92.7±8.6 minutes; $p<0.05$), lower REM density 1.5± 0.1 vs 1.9±0.1, and presumably as a result of the previous two factors, a lower REM index, a measure of the total number of eye movements (105.5± 9.3 vs 184.8±21.8; $p<0.01$). All other sleep variables were similar for the two groups. These observations constitute the first report relating individua differences in an enzyme involved in neurotransmitter metabolism to human sleep EEG parameters.

G. Arginine Vasotocin Alters REM Sleep in the Rat

Arginine vasotocin (AVT), a pineal peptide, has been reported to inhibit gonadotropin release, modify conditioned behavior, and enhance slow-wave sleep. The latter effect was particularly striking because of the remarkably small doses employed--10^{-6} picograms intraventricularly and 1 microgram intraperitoneally in the cat.[1] In order to further characterize the effects of this substance, we are reporting here a dose-response study on sleep and behavior in the rat.

Arginine (8) vasotocin was given intraperitoneally to 200 gm male Sprague Dawley rats in doses of 0.5 μg/kg (n=9), 50 μg/kg (n=9) and 500 μg/kg (n=5), and the EEG and EMG were monitored for 8 hours starting at 8:30 a.m. In a second study, 10 rats received 10 μg/kg at 8:30 a.m. and the following behavioral measures were done: righting reflex, an equilibrium test, grasping reflex, catatonia, and tail pinch reflex.

The lowest dose (0.5 μg/kg) had no effect on the sleep stages. The middle dose (50 μg/kg) decreased REM latency by 65 percent to about 29 minutes ($p<0.02$) and decreased ($p<0.008$) REM efficiency from 93.3± 1.0 (saline) to 86.9±2.0 (AVT). There was no effect on sleep latency or total sleep. When data were broken down into two hour periods, the major change was a 55% decrease in REM time ($p<0.02$) in the second two hours. During the first two hours of recording, the animals, when awake, assumed a characteristic quiescent crouching posture, and seemed less responsive to mildly disturbing stimuli such as air blown in the ear. The systematic behavioral tests did not reveal significant changes, although there was a trend toward remaining longer on the equilibrium bar.

At the highest dose (500 μg/kg) four out of the five rats died of pulmonary congestion and edema within a few hours.

These data indicate that 50 μg/kg has potent physiological effects. Although we did not confirm a report of increased slow-wave sleep in cats, our REM sleep data are reminiscent of the report of decreased REM in the first 5 hours. This also seems to confirm the observation that animals sleeping after AVT administration are easily aroused; when awake they appeared quiescent, yet did not have the neurologic deficits often associated with sedative/hypnotic agents.

H. The Effects of GABA Agonists and Antagonists on Sleep and Behavior in the Rat

It has been estimated that gamma-aminobutyric acid (GABA) functions as a neurotransmitter in 30 percent of mammalian synapses. Given its ubiquitous nature, and recent evidence that some of the effects of benzodiazepine anxiolytics and hypnotics (e.g., the anticonvulsant properties) may be mediated by GABA, it seemed appropriate to carefully assess the effects of GABA agonists and antagonists on EEG measures of sleep. We are reporting the results of administration of 2 and 4 mg/kg of picrotoxi (a GABA antagonist), 82 and 164 mg/kg of imidazole-4-acetic acid or IMA (GABA agonist) and saline on the sleep and behavior of the rat. All drugs were given at 8:30 a.m. intraperitoneally to 175 gm male Sprague-Dawley rats (approximately 9 in each group), and recordings were performed for 8 hours; because of the relatively short half-lives of these agents, data were also broken down into two-hour segments.

Analysis of the recordings revealed prominent seizure activity in three animals who received the high dose of picrotoxin; they were excluded from the sleep data. An ANOVA demonstrated that among the remaining animals there were significant differences in total sleep ($p<0.02$) and nonREM sleep ($p<0.02$) during the first two hours. A Neuman-Keuls analysis revealed that the high dose of IMA increased total sleep to 60.4±8.5 min compared to 23.3± 4.0 min for the high dose of picrotoxin ($p<0.01$). Saline control values for total sleep primarily represented alterations o nonREM sleep.

The effects of these agents on several behavioral tests were assessed in rats in each group. These measures included tail pinch, righting reflex, coordination on a balance bar and catalepsy (time remaining motionless with front feet placed on a horizontal bar). Of these, only the catalepsy test approached significance ($p<0.06$), indicating prolonged times with th high dose of IMA.

These data seem to confirm previous work suggesting that GABA agonists have sedative qualities. Further studies will be needed to assess the possible role of GABA in natural and pharmacologically-induced sleep.

I. Studies with the Delta Sleep-Inducing Peptide in the Rat

As part of a series of studies on possible circulating sleep factors, we have examined the effects of a "Sleep Peptide" marketed by Calbiochem (No. 567295), with an amino acid sequence identical to the "delta-sleep-inducing peptide." Originally this material had been discovered in the CSF of rabbits undergoing thalamic stimulation, and was reported to induc delta activity and spindles when administered to rabbits. It was later synthesized and reported to have similar actions.

In the studies reported here, the Sleep Peptide or artificial CSF (control) was administered intraventricularly to 200 gm male Sprague-Dawley rats. Drug administration was at 8:30 a.m., under conditions in which lights were on daily from 8:00 a.m. to 8:00 p.m. Recordings lasted 8 hours, and were interpreted in 30 second epochs as waking, nonREM and REM sleep.

Rats were studied with doses of 5.1 μg/kg (n=11) and 25.5 μg/kg (n=10). When data from the total 8 hour recording with the lower dose were examined, the only significant change was an increase in sleep latency (18.7±4.0 with control injection to 32.7± 5.5 min with active drug;

$p<0.009$) and a decrease in nonREM sleep (282.0±13.7 to 249.7±17.6 min; $p<0.04$). When the first two hours alone were considered, the only significant change was a slight decrease in the number of awakenings (6.4±0.5 to 5.1±0.7 min; $p<0.05$). At the higher dose, the only significant change was an increase in intermittent waking time (108.4±8.0 to 128.4±7.0 min; $p<.039$) over 8 hours, and an increase in REM-nonREM cycle length (14.0±6.2 to 27.2±5.4 min; $p<0.02$) in the first 2 hours.

In addition to the sleep studies, a pilot study of the effect of the same doses of the Sleep Peptide on the motor activity of 13 rats was conducted. No gross changes in motor activity were noted.

In summary, intraventricular administration of the Sleep Peptide to rats was found to have only minor effects on the sleep stages, and if anything they were in the direction of slightly disturbed sleep.

II. Hormones:

A. Regulation of Sleep-Related Growth Hormone and Prolactin Secretion

The study of pharmacologic manipulation of sleep-related growth hormone (GH) and prolactin (PRL) secretion has provided data on the roles of the serotonergic, noradrenergic and cholinergic systems in anterior pituitary regulation. The serotonin receptor blocker methysergide (2 mg PO q 6 h) was administered for 48 hours to normal young adults, and blood samples were drawn every 20 minutes during eight-hour sleep recordings. Methysergide administration was associated with an increase in several measures of sleep-related GH secretion. Mean peak concentrations of GH rose from 7.86±1.35 (SEM) ng/ml on control nights to 10.30±1.39 ng/ml with methysergide ($p<0.03$). Mean nocturnal PRL concentrations were decreased by 70.3%. In contrast to the increases in sleep-related GH secretion, daytime insulin-stimulated GH secretion was decreased 36.4% by methysergide.

These studies would seem to imply that: (1) serotonergic mechanisms may have an inhibitory role, and cholinergic mechanisms may have a facilitory role, in regulation of sleep-related GH secretion; and (2) data drawn from pharmacologic manipulation of daytime insulin provocative testing may lead to erroneous conclusions regarding physiologic control of GH secretion.

B. The Effect of Dexamethasone on Sleep-Related Growth Hormone Secretion in Congenital Adrenal Hyperplasia: Therapeutic Implications

Treatment of children with 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) with steroids has two goals: replacement of inadequate secretion of cortisol (and sometimes aldosterone) and suppression of oversecretion of adrenal androgens. In adults, and adolescents who have achieved full growth, it is most desirable to use long-acting steroids such as dexamethasone (DEX), given at midnight. The effects of DEX on nocturnal GH secretion need to be evaluated, however, particularly before use in children and adolescents who have not achieved full stature.

For this reason we have examined GH secretion and other endocrine measures during sleep in CAH patients receiving DEX in two different regimens.

The four females and two males who participated in the study ranged in age from 13 to 22. All but two (who were female) were salt losers. All patients received 0.5 mg DEX for six days each at 0700 and 2300 hours. Sleep studies were performed on night 5, and during a study in which several blood samples were drawn on night 6.

Examination of 24 hour excretion of 17 ketosteroids and pregenanetriol showed adequate suppression under both regimens in four of the six patients. In two subjects there was inadequate suppression with either regimen. LH was found to be secreted episodically throughout the night in a similar and normal manner with both regimens. Mean nocturnal FSH concentrations were also similar.

Examination of sleep-related GH revealed increased secretion during the first two hours of sleep when DEX was given at 0700 compared with administration at 2300. The areas under the curves of GH plots during this time were 1737±744 (SEM) and 666±359 ng/ml-min respectively ($p<0.05$). Following administration at 2300, there was a tendency for increased GH secretion later at night, which did not, however, reach significance.

Only two significant differences were noted on sleep EEG recordings made during the blood study: Following 0700 administration, there was a longer sleep latency (65.8±18.1 vs 24.2±7.9 minutes; $p<0.02$) and decreased nonREM sleep (266.8±34.4 vs 363.3±13.7 minutes, $p<0.02$). These observations were not borne out by the recording on night 5, and hence are of questionable biological importance. The possibility remains, of course, that DEX affects sleep differently when patients undergo the slight stress of an intravenous study compared to when they do not.

These observations suggest that administration of DEX at 0700 suppresses the adrenals yet permits sleep-related secretion of GH. Further studies of chronic use, and considerations of possible effects on somatomedins, will be necessary to further establish the safe use of DEX in children and adolescents.

C. LH-RH Administration and Human Sleep

Luteinizing hormone-releasing hormone (LH-RH), a decapeptide of hypothalamic origin, stimulates release of LH and FSH from the pituitary; in addition it may possess direct actions on the central nervous system. It has been shown, for instance, to cause mating behavior in estrogen-primed ovariectomized rats. Its administration has been reported to produce sexual arousal in human males with hypogonadism well before androgen levels reach the normal range. Because of these implications that LH-RH may possess central effects, and since gonadal hormones have been reported to influence sleep, we have explored the possibility that LH-RH administration may influence normal human sleep.

Subjects were 5 male and 5 female young adult normal volunteers. Females were studied during the follicular phase of the menstrual cycle. Following an adjustment night, each had sleep studies on two nights. On one night they received 100 mg of LH-RH in 500 cc of normal saline intravenously over an 8 hour period starting when the lights were turned out at 11:00 p.m. On the other randomly-sequenced night they received an equivalent volume of normal saline. The dose of LH-RH, while lower than those reported to produce human sexual arousal, has been shown to acutely raise serum LH and FSH.

Sleep EEG data revealed that LH-RH administration produced no change in the total minutes or percentage of the sleep stages, total sleep, or sleep latency. The subjects were also divided into two groups by sex, and once again there were no significant changes in sleep. It is concluded that low doses of LH-RH, sufficient to stimulate gonadal hormones, have no acute effects on normal human sleep. The possibility remains, of course, that higher doses or more potent analogues of LH-RH may have such actions.

D. Studies on the Role of Melatonin on Sleep in Rats

In the rat, the pineal hormone melatonin is synthesized and secreted in response to beta adrenergic stimulation, apparently only when two conditions are met: (1) there is darkness, and (2) it is the appropriate time of the circadian cycle. We have explored the effects of a large (833 μg/kg lP) dose of melatonin (chosen because it has been reported to elevate brain concentration of serotonin) and 20 mg/kg lP of propranolol (which blocks melatonin synthesis) on the sleep of the rat. Both drugs and saline were given to 175 gm Sprague Dawley rats at 7:45 a.m. and 7:45 p.m., and eight-hour recordings were performed. Lights were on in the laboratory from 8:00 a.m. to 8:00 p.m. daily.

In summary, it was found that melatonin given in the morning (the usual time of maximum sleep for the rat) decreased nonREM sleep by 48 min ($p<0.0009$) and enhanced intermittent waking by 35 min ($p<0.02$). When given at night, it had no significant effects. Propranolol produced rela tively little effect on nonREM sleep during the daytime, and enhanced it at night by 24 minutes ($p<0.04$). It decreased REM sleep at both times.

It should be noted that the drug-induced changes in nonREM sleep described here were significant, but small in comparison to circadian differences in sleep, and that the parenteral doses of melatonin were quite large. One possible interpretation of these results, however, is that in addition to its role as a zeitgeiber for a basic circadian rhythm, melatonin has direct effects on the nervous system (the role of light and darkness has been divided by some authors into these two kinds of activities). The "direct" effect of melatonin might be to enhance nocturnal behaviors (which in the case of the rat is increased wakefulness). In man, who sleeps at night, the result of administration would then be to enhance sleep, an effect reported by others. Further studies will be needed to explore this hypothesis.

E. Changes in Human Sleep Induced by Growth Hormone Administration

Although it has been known for some time that growth hormone (GH) is secreted in relation to sleep, the physiological significance of this phenomenon is unclear. It has been reported in animals that intraperitoneal injections of GH rapidly enter the brain and alter metabolism of biogenic amines, compounds which may play a role in processes such as thermoregulation, memory and sleep. We are reporting here the effects of GH administration on sleep, mood and memory in humans.

Eighteen normal young adult volunteers (16M, 2F) received IM injections of GH and saline 15 minutes before bedtime at 11:00 p.m. and sleep EEG, EOG and EMG were recorded for 8 hours. Doses of GH were 2 ($n=8$) and 5 ($n=10$) international units. In a companion study 15 subjects received injections of 2 or 5 units GH at 8:00 a.m., and had the following measures: a mood and behavior scale, 100 mm line measures of alertness and anxiety, serial learning lists, and the Buschke selective reminding test.

The lower dose of GH had no effects on sleep. The higher dose induced a 19% decrease in slow-wave sleep ($p<0.01$) and a 13% increase in REM sleep ($p<0.05$). In order to get a sense of GH concentrations related to these changes, serial blood sampling was done in two patients receiving 5 units. It was found that the injections resulted in peak concentrations of 60-80 ng/ml, dropping to less than 10 ng/ml by 7:00 a.m. Other measures, including sleep latency and total sleep, were unaffected. Neither dose, when given in the daytime, affected the mood and memory tests.

This study confirms previous work in animals, indicating that GH administration induces an increase in REM sleep, and also describes a decrease in slow-wave sleep. It raises the possibility that GH secretion may play a role in sleep-stage regulation.

F. Plasma Melatonin Concentrations During Human Sleep

In order to further define the relation of plasma melatonin to sleep, we have studied samples from six normal young adult volunteers (3M, 3F) and analyzed melatonin by means of a new, more sensitive mass spectrometric assay. Samples were drawn every 20 minutes from an indwelling venous catheter from 11:00 p.m. until 7:00 a.m., which coincided with the hours of darkness.

An analysis of variance revealed significant differences between subjects ($p<.0001$), different times of the night ($p<0.0001$) and state of consciousness ($p<0.009$). Concentrations rose during each of the 3 sequential time periods, progressing from 22.3 ± 4.4 pg/ml in hours 1 and 2, to 38.4 ± 2.8 in hours 3 and 4, to 56.6 ± 2.9 in hours 5 to 8. Analysis of the states of consciousness revealed that samples drawn during intermittent wakefulness were significantly ($p<0.01$) lower than during the rest of the sleep stages combined, and showed no significant relation to the individual stages.

Previous studies, using radioimmunoassays have indicated either a mean increase at roughly 2:00 a.m. or apparently random peak times; the present work seems to indicate gradual increasing concentrations of melatonin throughout the night. One report indicated no consistent relationship to individual stages of sleep, a finding borne out here. The significantly decreased concentrations during wakefulness, however, suggest that sleep, along with darkness and clock time, may influence melatonin secretion.

III. Major Findings: Clinical Disorders

A. Successful Separation of Depressed, Normal, and Insomniac Subjects by EEG Sleep Data

Data from all-night EEG sleep studies were used to distinguish normal subjects, primary depressed patients, and primary insomniac patients. In part 1, we compared 41 normal subjects, 56 depressed patients, and 18 insomniacs. In a univariate comparison with normal subjects, depressed patients showed less total sleep, longer sleep latency, more early morning awake time, more intermittent awake time, less delta sleep, less sleep efficiency, and shorter rapid eye movement (REM) latencies; compared with insomniacs, depressed patients showed greater early morning awake time, shorter REM latency, greater REM index, and greater REM density. Using multivariate discriminant analysis, 82% of the sample were correctly classified by diagnosis: 100% of the normal subjects, 72% of the depressed patients, and 77% of the insomniacs. Eight variables contributed to the multivariate separation of depressed individuals from insomniacs and normals: total sleep time, total recording period, sleep efficiency, sleep latency, early morning awake time, awake time, REM time, and REM %. When the discriminant functions were applied to a second group of 18 primary depressed patients, 82% were correctly classified as depressed. These results suggest that primary depressed patients and primary insomniac patients may show relatively characteristic patterns of sleep abnormality.

Part I: Classification of Normal Subjects, Depressed Patients, and Insomniac Patients by Discriminant Analysis: All 12 Sleep Variables

		No. (%)	
	Normal	Depressed	Insomniacs
Normal (N=41)	41 (100)	0 (0)	0 (0)
Depressed (N=56)	13 (23.2)	41 (73.2)	2 (3.6)
Insomniac (N18)	4 (22.2)	0 (0)	14 (77.8)

B. The "Switch Process" in Manic-depressive Illness: Circadian Variation in Frequency of Switches and Changes in Total Sleep Time

A "switch" was operationally defined as a rapid change in mood (change of three or more points on a 15-point scale) occurring in less than 24 hours.

Out of 75 bipolar manic-depressive patients, 35 showed 70 switches from depression to mania (D→M) and 37 switches from mania to depression (M→D). Both D→M and M→D switches occur predominantly in the morning (7 a.m.-3 p.m.). Among the D→M group, patients who switch at night (11 p.m.-7 a.m.) show significantly greater sleep loss for the next four days (Day 0 to +4), than if they switch in the morning (7 a.m. - 3 p.m.) ($p<0.01$, Newmann Keuls test). The evening shift does not differ from the other times. The M→D switches, however, fail to show a similar differentiation between the three time shifts with regard to total sleep time. These data indicate that time of switch may predict subsequent sleep changes in D→M switches.

In an unmedicated female bipolar patient fifty-eight all-night polygraphic sleep recordings were obtained during eight switches into and out of mania. While depressed she was hypersomniac and exhibited elevated REM sleep time and short REM latency. On four nights, she apparently switched into mania while asleep. The last recorded sleep stage in each case was REM sleep.

We have analyzed 144 switches (80 switches into mania and 64 out of mania) in another bipolar patient with regular 48-hour manic-depressive cycles. Switches into mania showed a sharp tendency to occur between 8 p.m. and 8 a.m. with peak incidence between 2 a.m. and 4 a.m. Similarly, switches out of mania occurred significantly more often between 4 a.m. and 6 a.m. than during other time periods. The frequency of distribution of these switches into and out of mania was significantly different from an expected random distribution (2XR chi square analysis with df=11). Switches during sleep: among the 80 switches into mania 37 occurred out of sleep (41%); among the 64 switches out of mania, 27 were associated with sleep (42%).

C. The Relationship between Changes in REM Sleep and Clinical Improvement in Depressed Patients Treated with Amitriptyline

EEG sleep recordings were obtained on consecutive nights from six hospitalized depressed patients before, during, and after treatment with amitriptyline. Amitriptyline significantly reduced time spent in Rapid Eye Movement (REM) Sleep and prolonged the REM Latency throughout the treatment period. Three patients who improved during treatment had a REM rebound when amitriptyline was discontinued, whereas three patients who did not improve showed no REM rebound.

D. Muscarinic Supersensitivity: A Possible Model for the Sleep Disturbance of Primary Depression

The sleep changes induced in eleven normal volunteers following the administration of scopolamine on 3 consecutive mornings resembles many of the abnormalities observed in the sleep of patients with primary depression: increased sleep latency, and reduced REM latency, total sleep time, and sleep efficiency. Furthermore, multivariate discriminant analysis, previously shown to distinguish the sleep records of depressed patients from those of normal controls and insomniac patients, selected the records from baseline nights as normal and those after scopolamine as predominantly depressed.

	Baseline	Scopolamine
Normal	11	4
Depressed or Insomniac	0	7*

$p < .005$ Fishers Exact Probability Test

* depressed 6
insomniac 1

These observations suggest that muscarinic supersensitivity in normals may function as a pharmacological model for the sleep disturbances of depression.

E. Faster Cholinergic REM Induction in Remitted Patients with Primary Affective Illness

We compared remitted patients with primary affective illness and normal control subjects on the "cholinergic REM-induction test." In this test we measure the speed with which REM sleep is induced by arecoline, a cholinergic muscarinic agonist administered intravenously during the second nonREM sleep period.

The control subjects were sixteen paid normal volunteers (9 males, 7 females, mean ± SD, age = 28.3 ± 5.4 years). They were compared with two groups of remitted patients with primary affective illness. The initial group (Group I) of thirteen remitted patients (4 males, 9 females, 12 bipolar and 1 unipolar, mean age 28.9 ± 6.9 years) was tested after all their regular medications had been discontinued for at least 2 weeks. To rule out potential confounding effects of prior psychoactive drug treatment or withdrawal on the arecoline response of Group I, we recruited a second group (Group II) of eight remitted patients who had never received any somatic therapy (4 patients) or had not received it within 4 months of the study (4 patients). The second group consisted of 6 bipolar and 2 unipolar affective patients (3 males, 5 females, mean ± SD, age = 29.1 ± 5.6 years). All patients met research diagnostic criteria for primary affective illness and had been in clinical remission for at least 3 months at the time of the study.

Arecoline induced REM sleep significantly faster in both patient groups than in controls. Following arecoline 0.5 mg, mean Inf-REM$_2$ latency was 38 min in normals, 11 min in Group I, and 15 min in Group II ($p<.01$, $H=10.22$, df-2, Kruskal-Wallis one-way analysis of variance). The mean Inf-REM$_2$ duration was significantly shorter for both Group I ($p<.01$) and II ($p<.025$, Mann-Whitney U-test) than for normal controls. Arecoline 1.0 mg was administered only to normal controls and Group I; again, the patients entered REM significantly faster than controls (7 minutes versus 19 minutes, $p<.01$, Mann-Whitney U-test).

G. Catecholamine Depletion: Sleep and Mood Changes in Man Mimic the Bipolar Switch Process

Alpha-methyl-para-tyrosine (AMPT, 3 gm/d) was administered in a double-blind fashion to 9 normal volunteers (mean age 24) for 3 days. During AMPT administration, such ratings of mood indicated significantly increased depression, while sleep studies indicated hypersomnia and reduced REM sleep. During withdrawal from AMPT, mood ratings suggested hypomania and sleep studies indicated a transient hyposomnia. Co-administration of lithium (900-1200 mg/day) attenuated the withdrawal hypomania. These results suggest that AMPT in normals mimics the bipolar "switch" process from depression to mania.

H. Sleep of Bipolar and Unipolar Depressed Patients Compared

The sleep of 36 normals, 36 unipolar patients, and 22 bipolar patients was compared. The only sleep variable which significantly differed between unipolar and bipolar patients was REM efficiency. Bipolar patients showed a significant reduction compared with unipolar patients (82% ± 3 versus 91% ± 1, $p<.001$). In addition, "hypersomnia" (defined as more sleep than the age-corrected normal mean) was more prevalent among the bipolars than the unipolars ($p<.05$, $X^2 = 3.81$).

I. Schizophrenia:

1. Schizophrenia

Eight actively ill schizophrenics and eight nonpsychotic controls were deprived of REM sleep by the awakening method for two nights. Sleep patterns during five post-deprivation nights were analyzed by a variety of multivariate techniques.

Data suggest that actively ill schizophrenics are less likely than control psychiatric patients to exhibit a normal REM rebound. They require fewer awakenings than controls to achieve REM deprivation. They show little or no change in REM time or REM% during recovery as compared with baseline, and, compared with controls, have significantly less REM time, REM%, and change in REM time and REM% on early post-deprivation nights.

The two patient groups also differed in their pattern of stages III and IV during recovery. Considerable overlap existed in REM compensation between actively ill schizophrenics and controls. Additional information suggests that REM compensation may be related to Rod and Frame testing: the more field independent a subject is, the better REM compensator he is.

2. 5-Hydroxytryptophan and Schizophrenia

5-Hydroxytryptophan was studied at doses of 200-60000 mg/24 hr with MK486 in 18 chronic schizophrenic patients. EEG sleep studies revealed: (a) REM sleep was reduced at all doses; no rebound was found with 5HTP withdrawal; (b) REM latency was increased at all doses and (c) NREM sleep was increased at low doses, decreased at high doses, and decreased still further during withdrawal.

J. Sleep in Gilles de la Tourette's Syndrome

The sleep of 6 Tourette patients (drug-free and while taking haloperidol) was compared with 9 normal volunteers. The untreated patients had 30% less delta sleep, which returned to control values when they received haloperidol.

K. A Case of Sleep-walking and Somnambulistic Homocide Associated with Psychotropic Drugs

The patient is a 44 year-old white female who had a history of one sleep-walking episode in adolescence but none after that until age 40. She also had had enuresis until age 16. Except for brief psychiatric treatment at age 16 for enuresis and anxiety, she had no psychiatric history until age 39, when she developed a florid psychosis with persecutory symptoms. She was diagnosed as schizophrenic, hospitalized for 2 weeks, and treated with phenothiazines. A year later she experienced a return of symptoms and resumed treatment on an outpatient basis. Because of insomnia, she received thioridazine and Triclos (a chloral derivative) at bedtime. At 3 a.m. on the third night of this regimen, she arose from her bed and stabbed her daughter to death, possibly while asleep or in a confused state. She was found to be hallucinating and delusional when examined the next morning and diagnosed as suffering from paranoid schizophrenia. At her trial she was found innocent on the grounds of insanity and hospitalized under court order.

On phenothiazines and psychotherapy, her psychosis slowly cleared. However, she was noted to sleep-walk 2-4 times per week. Physical investigations, which eventually included EEG's with nasal-pharyngeal leads, a CAT scan, and a pneumoencephalogram, were all normal. Dilantin and tricyclic antidepressants had no effect on her sleep-walking.

After 4 years in the hospital, she was transferred to our unit, and thioridazine 300 mg q.h.s. was discontinued. During a one-month drug-free period, 11 all-night sleep EEG's along with continuous video monitoring were carried out. There were no sleep-walking episodes. However, when treated with thioridazine 300 mg q.h.s. and chloral hydrate 1000 mg q.h.s. she sleep-walked on 5 of the 6 nights recorded. On thioridazine 300 mg q.h.s. alone, she walked one night in four; on chloral hydrate 2000 mg alone, she did not walk during three nights of recording. All sleep-walking episodes occurred out of slow-wave sleep.

These results suggest that she sleep-walked only on psychotropic medications, particularly thioridazine. This case is similar to those reported by Huapaya (Am. J. Psychiatry 133: 1207, 1976) of somnambulism secondary to bedtime doses of methaqualone in two cases, chlorprothixene in another, and the combination of methaqualone and thioridazine in the fourth. In all cases sleep-walking could be abolished by either reducing or eliminating the bedtime dose. Two of the cases had childhood histories of sleep-walking, as did our own. As well, Flemenbaum (Am. J. Psychiatry 133:570-572, 1976) has reported cases of pavor nocturnus, another disorder associated with stage 4 sleep in patients receiving large bedtime doses of tricyclics or antipsychotics. The mechanism of this phenomenon, which may not be uncommon, is unknown. We are presently analyzing our patients' sleep EEG's.

IV. Major Findings: Normal Studies

A. Effects of Sleep Stage and Stimulus Intensity on Auditory Evoked Responses

The purpose of this study was to evaluate the effect of sleep stage and stimulus intensity on the auditory evoked response (AER). Nine normal volunteers (age 18-22; 6M, 3F) slept in the sleep lab for three nights each; the first night was adaptation and was not included in any calculations. Four intensities of clicks (50, 60, 70 and 80 dB) were generated by the LINC computer in pseudo random order. EEG was sampled at 250 Hz for 1 second and AER was computed on line for each intensity for each minute of the night. Each minute was visually scored for sleep stage. AER from the minutes of each sleep stage were then averaged. AER amplitude tended to increase little from 50 to 80 dB in waking subjects but increased markedly in sleeping subjects in stages 3 and 4 ($p<0.05$, 2-way analysis of variance). Rapid eye movement (REM) and stage I sleep had small amplitude AER's in comparison with other sleep stages.

Individuals who showed decreases in amplitude at high intensities while awake slept significantly longer during the experimental night ($r=.76$, $p<0.05$). The loss of inhibitory mechanisms during stages 3 and 4 is well known. It is therefore not surprising to see the disappearance of the paradoxical AER amplitude reduction at high intensities and a more linear EP amplitude/stimulus intensity function; stage 1, 2 and REM sleep resembled the waking state. Of interest was the finding that waking AER amplitude/stimulus intensity functions predicted sleep duration in this noisy environment. This suggests that individual differences in sleep duration may be related to differences in inhibition of sensory processing.

V. Major Findings: Animal Behavior

A. Deprivation of Rapid Eye Movement Sleep and Nesting Behavior in Rats

The effects of REM deprivation for four days on nesting and locomotor activities were studied in male rats. Four groups of animals were tested. (a) Experimental animals (SP), deprived of REM on a small (6.5 cm) pot; (b) Large pot (LP) control, maintained on large (12.5 cm) pots; (c) stress group, forced swimming for 1 hr/day; (d) normal controls. The results indicate, as predicted, increased utilization of nesting material by the experimental (SP) group in the post-REM deprivation period as compared with normal controls (464 cm for SP, 94 for normals, $p<.05$). Nevertheless, the SP group did not differ significantly from LP (356 cm) or stress (133 cm). Since LP does suffer some REM deprivation, though less than SP, the results suggest that increasing levels of REM deprivation may increase a drive-related behavior in rats.

B. EEG Documentation of a Method for Sleep Depriving Rats

There is a need for a relatively simple method of sleep deprivation which is supported by EEG data and which can process a number of animals at once with a minimum of labor. We shall describe such a technique, which consists of keeping rats awake by maintaining them in slowly rotating cylinders.

Rats were placed in two Nalgene cylinders (68.6 x 35.6 cm), each of which is divided into six compartments. An electric motor causes them to rotate at 3 RPM. A strip of tubing on the floor of each compartment provides an obstruction over which rats must walk, so that they cannot slide along.

In order to document the state of consciousness of rats undergoing this procedure, we recorded the EEG and EMG of four rats in the moving cylinders for 24 hours and compared them to seven animals in a stationary cage. The moving cylinder reduced sleeping time from 47.0% of the 24-hour period to only 3.8% ($p<0.001$). The small amount of sleep which did occur was made up of both REM and NREM sleep. The percentages of total sleep time did not differ between the two conditions. Thus, there was no selective effect on one particular sleep stage, and instead total sleep time as a whole was markedly reduced.

C. Signal Anlysis of the Rat Sleep EEG: The Application of Continuous Frequency Analysis

Several automated systems for scoring rat sleep have been developed, usually employing signal processing by filtering into bands of particular interest, and then making a determination of either signal amplitude or rectified signal amplitude. Although empirically these are clearly very functional, they do not provide a measure of signal power or energy. Power measures may be derived from spectral analysis using the Fast Fourier Transform, but this approach is costly and requires dedication of relatively sophisticated equipment. We have developed a relatively simple hardware system which determines sleep state by a measure which approximates signal power. It is based on Parseval's relation, which shows that the integral of the square of a signal filtered by a bandpass filter is a measure of the signal's power at those frequencies. In practical terms, the signal is filtered, squared and integrated. The slope of the integrated output provides a measure of activity in the selected bands.

We have constructed a scorer employing analysis of bifrontal delta and fronto-occipital delta and theta activity, and 20-200 Hz nuchal EMG activity. These values are input to a logic network, which provides a two bit output coded to recognize waking, nonREM and REM sleep in 10 second epochs.

The validity of this method was tested by comparing five two-hour recordings as interpreted by the system and an experienced human reader who read each record twice. Kappa values were highly significant {0.76± 0.01 (first reading) and 0.73±0.01 (second reading)} indicating the validity of this system.

D. Sleep in Rats with Bilateral Habenular Lesions

The diencephalic habenula nuclei (HBN), which receive afferents from several limbic structures, are thought to send an inhibitory pathway to the dorsal raphe nuclei (DRN). Thus electrical stimulation of the HBN has been reported to decrease DRN cell firing, and bilateral lesions of the HBN may lead to a 60% increase in 5 HIAA content of the DRN (manuscript in preparation). These lesions have been associated with increased daytime motor activity in rats (manuscript in preparation). Because of longstanding interest in the role of the raphe in sleep regulation, we have examined the effect of bilateral HBN lesions on sleep in the rat.

Ten male 200 gm Sprague Dawley rats received bilateral radio frequency lesions of the HBN, and 11 had sham operations. They were then implanted with EEG and EMG electrodes, and 8 hour recordings starting at 8:30 a.m. were performed one week later. Analysis of the data revealed no significant changes in sleep latency, total sleep, or any sleep stage measure.

A lesion producing relatively drastic changes in dorsal raphe cell activit might well be expected to influence sleep, and it is thus surprising that no such change was found. Alternative explanations for this might be that (1) lesion effects are seen only at other parts of the circadian cycle; or (2) during the week between lesioning and recording, the nervous system develops tolerance to perturbations from altered serotonin metabolism. On argument in favor of the latter hypothesis comes from reports of tolerance to the effects on sleep of the serotonin synthesis inhibitor parachlorophenylalanine.

VI. Studies of Human Memory:

A. Effects of Arecoline or Scopolamine Prior to Learning.

We determined the effects of arecoline and scopolamine on categorized serial learning in normal volunteers, all of whom were college students or recent college graduates.

The results indicated that arecoline (4 mg) significantly reduced the number of trials to criterion as compared with saline (mean 3.75 vs. 5.18 trials, $p<.01$). In contrast, the mean number of trials following scopolamine (0.5 mg) was 6.35 ($p<.01$, compared with 5.18 on methscopolamine-saline control). Administration of arecoline 6 mg, however, reversed the effects of scopolamine 0.5 mg (mean number of trials was 4.92, which was significant, $p<.01$, compared with scopolamine alone).

We also found that the major effects of arecoline and scopolamine were seen in naturally "poor" performers rather than "good" performers. The correlation between baseline, placebo performance and change on arecoline was $r=0.93$, $p<.001$, indicating that greater improvement was seen in those subjects who took the most trials to criterion on baseline; for scopolamine, the correlation was $r=0.5$, $p<.05$, indicating that the greatest decrement in performance was seen in those who took the most trials to criterion.

B. Effects of Arecoline Following Learning

We showed that administration of arecoline immediately after learning facilitates later recall. Eight subjects (5 males, 3 females, mean age 24) were tested on two nonconsecutive days. Prior to the experiment, an intravenous needle was inserted in a forearm vein and attached to a ten-foot catheter extending out of the room so that drugs could be administered without the awareness of the subject. Following administration of methscopolamine (0.3 mg iv), subjects heard a list of words, nine groups of five words each. Three of the nine groups consisted of highly related (associated) words, three groups of weakly associated words, and three groups of randomly associated words. The groups were presented in order: first a highly related group, then a weakly associated group, then a randomly associated group, until all nine groups had been presented. After listening to each group of five words, subjects were asked to think of and say a word which defined or organized that word cluster. Immediately at the end of the session, subjects received a twenty minute infusion of either arecoline (2 mg in 20 ml of saline) or placebo (20 ml saline). Recall testing began 1 hour following the onset of the infusion. Subjects were tested first under free recall conditions and, then, under "cued" recall conditions (after having been presented with their own, self-generated cue word).

Compared with placebo infusion, arecoline significantly improved recall of the presented word; all three types of word sets--highly associated, weakly associated, and randomly associated--were better recalled ($p<.05$,

$F=7.8$, $df=1,7$). This was true under both free and cued recall conditions. Interestingly, the arecoline effect appeared to be mediated by an increase in the number of clusters recalled ($p<.01$, $F=13.6$, $df=1,7$) rather than an increase in the number of words per cluster. While cued recall was better than free recall ($p<.001$, $t=29.6$, $df=1,7$), and highly associated word sets were better recalled than weakly or randomly associated words, the facilitory effect of arecoline did not interact with these factors.

C. Effects of Choline Chloride on Human Learning.

To study the effects of choline chloride in man, we administered an elixer containing 10 gm or placebo on separate days. Ninety minutes later, we began the session with two different tests: uncategorized serial learning and a selective reminding task.

Choline administration improved performance on both tests. On the uncategorized serial learning tasks, subjects learned to recite ten unrelated words in correct order; following each trial, the entire list was repeate by the experimenter. The number of trials to criterion was 5.7 ± 0.7 after choline and 6.1 ± 0.9 after placebo ($p<.05$, two-tailed t-test). On the selective reminding task, subjects learned a 12 word list of words, half of which were high image words (such as table) and half of which were low image words (such as virtue). Following each trial, the investigator reminded the subject of the words missed on the last trial, but did not repeat the words correctly remembered. As might be expected, high imagery words were learned in fewer trials on placebo than low imagery words, and choline did not alter the number of trials required for high imagery words. Choline administration, however, significantly reduced the number of trials required to learn low imagery words from 9.1 ± 0.9 to 6.8 ± 0.5 ($p<.01$).

The effects of choline were significantly correlated with performance on placebo, indicating a greater effect in "poor" performers than "good" performers. The change induced by choline was significantly correlated with performance on placebo on the uncategorized serial learning task ($r=0.59$, $p<.05$, $N=10$) and on low imagery words of the selective reminding task ($r=0.86$, $p<.01$, $N-10$).

D. The Effects of Lecithin on Memory and Plasma Choline Levels in Normal Volu teers

A pure grade of lecithin (Phospholipon 100 - about 95% phosphatidylcholine was administered to 15 normal volunteers in doses of 20, 40, and 60 gm. A dose-dependent elevation of plasma choline was noted, which persisted for at least 12 hours. The highest dose produced some nausea and GI symptoms. No overall change in memory and learning was noted. The "poor performers, however, again showed a significant dose-dependent increase in performance at 20 and 40 gm as compared with placebo.

E. Cholinergic Therapies in Alzheimer's Disease

Preliminary data fails to show a significant effect of lecithin in patients with clinically defined Alzheimer's disease. Nevertheless, it does appear that the combination of lecithin and THA (an oral anticholinesterase) may improve cognitive performance. Further studies are underway.

VII. Pupillometry Measures in Patients with Primary Affective Illness

We used the ocular miotic response to a cholinomimetic as the physiological measure of cholinergic muscarinic sensitivity. Pilocarpine acts postsynaptically on the iris sphincter as shown by the persisting miotic potency even after retrobulbar anaesthesia (which blocks presynaptic release of acetylcholine).

Twelve paid normal volunteers and eight remitted patients with primary affective illness were studied. All patients were drug free for at least 2 weeks prior to study. Subjects with a history of glaucoma or who wore contact lenses were excluded.

Pupils were visualized on a split screen with two infrared television cameras, one for each eye, illuminated by infrared lighting on a split screen. Recordings were obtained at baseline and 15, 30, 45, 60, 75, 90 and 120 minutes after subconjunctival instillation of one drop (0.04 ml) of pilocarpine.

Mean baseline pupillary diameter (combined average of both eyes and different pretreatment baselines) was 6.5 ± .54 mm (mean ± standard deviation) in normals and 6.36 ± .47 mm in patients (p = NS). A two-way ANOVA with repeated measures showed a highly significant difference between groups for both the 1/16% ($p < .001$) and 1/8% doses ($p < .008$). Pilocarpine produced a faster, more intense and longerlasting miosis in patients as compared to normals. Our data showed essentially little or no response in normals to the 1/16% dose and about 0.91 mm constriction to the 1/8% dose. The response to the 1/16% dose in patients exceeded the response of normals to 1/8% pilocarpine.

The results of the present cholinergic miosis test, like that of the cholinergic REM induction test, are further evidence for a state independent cholinergic involvement in affective illness. Although pilocarpine exerts its effects predominantly as a muscarinic receptor agonist, we do not exclude the possibility that noncholinergic systems are also involved in either affective illness or in the pupillary response.

VIII. Thermoregulation in Patients with Primary Affective Illness

The regulation of body temperature may be another CNS function which may be under the partial control of acetylcholine. We studied the hypothermic response of arecoline in a group of 17 normals and 10 primary affective patients in complete remission who were drug free for more than

2 weeks. Arecoline was administered as a slow IV drip (.066 mg per min, total dose = 2 mg every 10 minutes). There was no difference between normals and patients with respect to the hypothermic effect of arecoline.

Our data suggests that patients with primary affective illness appear to be hyper-responsive to pharmacological challenge with cholinergic agonists in two out of three measures tested; i.e., REM sleep induction, and pupillary miosis. They did not differ from normals with respect to the hypothermic effect of arecoline. The regulation of temperature, like blood pressure, is, of course, a very complex central and peripheral nervous system function. Thus, it may not be an appropriate physiological system to measure specific cholinergic effects.

Our aim has been to develop pharmacological challenge strategies utilizing physiological endpoints that could be objectively measured. Our data strongly supports the existence of a hyper-responsive cholinergic system in affective illness, both during the depressed and euthymic (remitted) states.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00098-06 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study of Children and Grandchildren of Patients with Depressive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Cytryn, M.D.	Medical Officer	BP	NIMH
Other:	Donald H. McKnew, M.D.	Medical Officer	BP	NIMH
	Walter Sceery, J.D.	Social Worker	BP	NIMH
	Joel R. Hamovit, M.S.W.	Research Social Worker	BP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.4	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness, and to identify biological markers of a genetically-transmitted predisposition to affective disorders, and to study the interaction between genetic predisposition and environmental factors in children with affective disorders. Our present project is a refined replication of our pilot project reported last year. The major changes involve the inclusion of two control groups as well as a blind interviewer and rater.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness; 2) to identify biological markers of a genetically-transmitted predisposition to affective disorders; and 3) to study the interaction between genetic predisposition and environmental factors in children with affective disorders.

Methods Employed: We are studying three groups of children: 1) 20 children of parents hospitalized at NIMH with a primary affective illness; 2) 10 children of patients hospitalized at NIH because of a physical illness and screened for any affective disorder; and 3) 20 children of "normal" parents with no known physical or mental disorder. All children are seen twice, four months apart. 4) There was also a comparison group of 11 children of parents with chronic physical illness, not screened for mental illness.

A social worker obtained at each visit a history from the parents and administered the Achenback Scale for childhood psychopathology.

At each visit, the child had a psychiatric interview. The interview was conducted by a blind interviewer and rater, while another investigator observed through a one-way mirror. The interviews were structured, and followed a standard inventory of topics aimed at eliciting various types of psychopathology such as affective disorders, anxiety reaction in childhood psychosis, delinquency and minimal brain dysfunction. A portion of the interview is aimed at eliciting fantasy material by the use of free drawings, story telling, and free reporting of dreams, T.V. stories, and movies. Other methods used in child psychiatry such as the three wishes and the Despert fables were also employed. Following the interview, the children were rated on the CPRS scale of the ECDEU as well as on a global rating scale for affective disorders constructed for this purpose and used by the investigators for many years. All of the above mentioned procedures pose no hazards for the children. In addition, after each visit, the children were rated on the Weinberg criteria for depression in children.

Major Findings: The children in the manic-depressive group had significantly more depression on all interviews than the two control groups, according to the Weinberg Criteria. The children in the two control groups did not differ from each other in the incidence of depression. However, both control groups had significantly less depression than the children of manic-depressive parents.

As for the comparison group, of the 10 parents with chronic physical illness 7 had also a concommitant major mental illness, mostly affective and in the "depressive spectrum". The 11 children in this group did not differ significantly from the children of the manic-depressive parents in the incidence of depression.

Significance to Biomedical Research and the Program of the Institute: This study confirms the findings in our pilot study about the high incidence of significant depressive illness in the children of parents with affective disorders. The question still remains, however, whether the depression in the offspring is genetically determined or mainly due to environmental factors. The answer to this question may have to await the finding of a biological marker for affective disorders.

Proposed Course of Project: The 20 children of manic-depressive parents and 20 matched control children, are currently being reevaluated using the above mentioned criteria and the revised DSM-III diagnostic system.

Publications:

Cytryn, L., McKnew, D H. Jr., Bartko, J. J., Lamour, M., Hamovit, J. and Bunney, W.E., Jr.: Offspring of Patients With Affective Disorders II. Submitted for publication in the British Journal of Psychiatry.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00100-05 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Psychological and Biological Aspects in Childhood and Adolescent Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
	William E. Bunney, M.D.	Chief	BP	NIMH
	Monte S. Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BP	NIMH
	Christy L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS

COOPERATING UNITS (if any)

Section on Experimental Therapeutics, LCS, DCBR, NIMH; Communicative Disorders Program, NINCDS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Office of the Chief; Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.75	1.45	3.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An inpatient program with selected overnight stays, for childhood and adolescent neuropsychiatric disorders is ongoing. The condition currently under study is that of hyperactive children (HAC). Pharmacological compounds under study in these disorders include methylphenidate, amphetamine, piribedil, L-dopa, tryptophan, and Mianserin. Pharmacokinetic studies with clinical responses are ongoing. Central neurotransmitters and their metabolites are being studied in plasma and urine. Amphetamine half-life in children is about one-third that of adults. Behavior and motor activity responses to d-Amphetamine occur during the absorption phase as determined by serial plasma amphetamine following a single dose. Urinary 3-methoxy-4-hydroxy-phenyl glycol shows a time-related decrease during treatment with d-amphetamine; homovanillic acid is unchanged. Piribedil is safe but clinically ineffective in HAC. L-dopa is minimally clinically effective in HAC.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of this program are broad. In the most basic sense, the objective is to gain new knowledge of the central nervous system (CNS) of children and adolescents with especial reference to maturational changes. Compared to the neurobiology known in adult neuropsychiatry, much less is known regarding the neuropsychiatric disorders of children.

Several principles are important to keep in mind in the study of children versus adults: 1) morphological brain maturation is age related, i.e., the reticular activating system, of considerable importance in psychobiology, is not fully myelinated until ages 10 to 20; 2) children may have more rapid and clear pharmacological responses, both therapeutic and toxic; 3) no clear history of pre-adolescent "addiction" to compounds, i.e., amphetamine, which can effect unequivocal addiction in adolescents; 4) most behavioral symptoms in children that respond to pharmacological treatment have more of an apparent organic contribution than those of adults, i.e., motor hyperactivity, enuresis, poor attention span in children versus complex emotional and cognitive aberrations in adults; 4) clinical symptomatology is more variable and fluctuating in children than usually seen in adults, i.e., rating scales for endogenous depression in adults are more easily constructed to measure a "constellation" than is the case for childhood depression; well formed delusions and hallucinations are not so common in young psychotic children as in adults.

A number of studies, pharmacological and otherwise, have been undertaken to elucidate cerebral metabolism in specific conditions.

Methods Employed: An inpatient and day patient program for children and adolescents, involving selected overnight stays, has been established on the 2 West Nursing Unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties have been admitted in order to study a carefully defined population of HAC. Children and adolescents with other conditions have been studied.

All children are thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Children also receive a psycholinguistic examination in collaboration with NINCDS. Neurological examinations are scored carefully according to a rating scale (PANESS). Several clinical and behavioral rating instruments have been utilized.

Pharmacological compounds, both standard and those previously unused in children, are being studied. Serial plasma pharmacokinetic data are being generated for methylphenidate and amphetamine. These data are studied in conjunction with motor activity, behavior, cognition, speech, temperature, and cardiovascular response. Piribedil, a specific dopamine (DA) agonist, and L-dopa has been given to HAC. Both Mianserin, a norepinephrine (NE) agonist, and tryptophan (TP), a precursor of serotonin (5HT), are being administered to HAC as clinical trials.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. At any time the instrument can be read into a computer for a print-out. Behavioral changes in HAC are measured via Conners' Teachers' Rating Scale (CTRS). Cognition is measured by a continuous performance task (CPT) in which errors of omission and commission can be scored in terms of differing time intervals. Time intervals can also be increased or decreased in relationship to accuracy of response.

Amphetamine is measured by a radioimmunoassay (RIA) and gas chromatograph-mass spectrometry (GC-MS). Biochemical studies include 24-hour urine collection to study homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG). Plasma pharmacokinetics of pharmacological compounds are being ascertained. Plasma NE, MHPG, metanephrine (NMN) and vanillylmandelic acid (VMA) changes as they relate to plasma amphetamine levels have also been studied. Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Average Evoked Response (AER) studies are being conducted as they relate to HAC in drug-free and treated conditions. Psycholinguistic changes are also studied in relation to amphetamine, piribedil, and TP effects. Paired associate learning has also been assessed in different drug conditions. Chronic effects of d-amphetamine (2 weeks) are being studied with regard to pharmacokinetics and clinical response, particularly in terms of an evidence for tolerance or supersensitivity and effect on catecholamine metabolism (as manifested by changes in plasma NE and dopamine-beta-hydroxylase (DBH) and platelet 5HT and monoamine oxidase (MAO).

Major Findings: Significant correlations exist between community teachers' and NIH teachers' CTRS ratings of behavior.

Serial plasma pharmacokinetic data indicate that children reach a peak serum level of amphetamine within 3-4 hours (h) of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 ± 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data is highly replicable. Clinical responses have also been studied after use of sustained release capsules. This preparation produces a slower rate of absorption and a more plateau-like, longer lasting peak level, but does give a prolonged clinical response. Socially appropriate behavioral change, increased attention span, and concentration ability is maximal at 1-3 h after administration of a single dose (0.5 mg/kg) of d-amphetamine during its absorption phase. This data indicates that the clinical changes may be related to depletion of catecholamine stores, to replacement by a "false" neurotransmitter metabolite of amphetamine, or to alteration in receptor sensitivity. Higher single doses (1.0 mg/kg) effect similar clinical responses, but less so. Piribedil is safe, but clinically ineffective for HAC.

Studies of urinary metabolites indicate that day and night excretion of MHPG and HVA are not significantly different; however, d-amphetamine after eight days is associated with significantly lower MHPG levels and the data indicates that behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged.

HAC are not significantly different from normal controls with regard to plasma NE and DBH but do have significantly more neurological soft signs by PANESS examination. Plasma NE is significantly correlated with anxiety as well as being significantly elevated at 2-3 h following a single oral dose of d-amphetamine.

Studies evaluating CPT are now underway; amphetamine is effective and piribedil and L-dopa are minimally so. AER studies are preliminary. HAC with higher levels of soft signs have more abnormal EEG's, more minor physical anomalies, lower full scale IQ (WISC-R) and a greater number of errors on the Bender. Data from psycholinguistic evaluations are being prepared for publication.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly HAC, have been considerably studied in the last few years, there are many diagnostic, psychopharmacological, and psychobiological questions to be answered. Many studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards HAC, obsessive-compulsive children, enuretics, Gilles de la Tourette's syndrome, Anorexia Nervosa, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and amphetamine give positive responses in 80% of well diagnosed HAC, the pharmacokinetics and metabolism of these drugs had not been closely studied in children. The relationships between such basic pharmacological knowledge and clinical effects have been understudied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in understanding the biological contributions to childhood neuropsychiatry.

Proposed Course of Project: We plan to conduct this program over an indefinite period.

Publications:

Caine, E.C., Margolin, D., Brown, G.L., and Ebert, M.H.: Gilles de la Tourette's syndrome, tardive dyskinesia, and psychosis in an adolescent. Am. J. Psychiatry 135: 241-243, 1978.

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Mikkelsen, E.J., Brown, G.L., Millican F.K., Rapoport, J.L.: Neurologic status in hyperactive, enuretic, encopretic, and normal children. J. Am. Acad. Child Psychiatry, in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00101-04 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Childhood Enuresis: Clinical Response to Placebo, Imipramine and Methscopolamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Wallace B. Mendelson, M.D.	Research Psychiatrist	BP	NIMH
	J. Christian Gillin, M.D.	Research Psychiatrist	BP	NIMH
	Richard J. Wyatt, M.D.	Director	SMR	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)
Division of Special Mental Health Research, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 00	PROFESSIONAL: 00	OTHER: 00

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project has been discontinued.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00102-03 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Lithium Response in Children of Lithium Responding Manic Depressive Parents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald H. McKnew, M.D.	Medical Officer	BP	NIMH
Other:	Leon Cytryn, M.D.	Medical Officer	BP	NIMH
	Monte S. Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BP	NIMH
	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
	Elliot S. Gershon, M.D.	Chief, Section on Psychogenetics	BP	NIMH
	Joel R. Hamovit, M.S.W.	Research Social Worker	BP	NIMH
	Walter Sceery, J.D.	Social Worker	BP	NIMH

COOPERATING UNITS (if any)
Laboratory of Psychology and Psychopathology, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of lithium in emotionally disturbed children having at least one manic-depressive lithium responsive parent will be examined. Thirty children, ages 6-12, of both sexes, having psychiatric symptoms that are in some way incapacitating and are of major concern to the child, parent, or teacher will be selected. Children will be given lithium and placebo, in a double-blind, crossover design for 16-18 weeks. Behavior ratings will include: parent's and teacher's assessment, psychophysiological measures, child interview, and cognitive testing. The lithium administration will be monitored by an "unblind" co-investigator.

Project Description:

Objectives: 1) To test the hypothesis that some of the offspring of lithium responding parents will prove clinically responsive to lithium. 2) To test the hypothesis that children with an affective and/or cyclic component to their psychopathology will be better responders. 3) To see if the response to lithium may possibly be a genetic marker for manic-depressive illness. 4) To identify the children at risk with early prodromal disorders who respond to lithium, prior to the outbreak of manic-depressive illness.

Methods Employed: The 30 patients will be admitted to the study upon recruitment from the NIMH Outpatient Department (OPD) as well as from the local psychiatric community. The fact that the parents are indeed lithium-responding manic-depressives will be independently verified on the basis of SADS interviews and RDC diagnoses.

The patients will be selected on the basis of having sufficient psychopathology for it to be incapacitating and to be of concern to their parents and/or the school authorities. Any children with significant brain damage and/or an IQ below 80 will be excluded from the study. The patients will be ages 6-12 of both sexes, and with no regard for their socio-economic status. These patients will be admitted to the Outpatient Clinic for the duration of the study. Patients of special interest may be retained in the OPD for follow-up treatment and study. Other patients will be returned to their previous source of care or referred to local psychiatrists, as needed.

Their work-up records will be handled as described in the Handbook for Staff Physicians.

Admission Work-Up:

Parents: A structured history will be obtained. In addition, the parents will be requested to fill out the following questionnaires: 1) Deviant Behavior Inventory (DBI); 2) the Achenbach Scale.

Patients: A diagnostic evaluation will be obtained including a structured interview and a routine physical exam. On the basis of all available information, a clinical diagnosis based on DSM-III will be made. The following rating scales will be filled out: 1) Children's Psychiatric Rating Scale (CPRS of the ECDEU); and 2) Children's Affective Rating Scale (CARS).

Blood will be drawn for creatinine, BUN and T_3, T_4 and TSH to rule out renal or thyroid pathology. These tests will be repeated at the end of the study.

The physiological test, Average Evoked Response (AER), will be administered.

Teachers will fill out the brief Conners' Teacher Rating Scale.

The thirty patients will be randomly assigned to 2 groups (I and II). Group I will be treated as follows: Approximately 4 weeks placebo, 4 weeks lithium, 4 weeks placebo, 4 weeks lithium. The procedure will be reversed in Group II.

Nature of Procedure: Lithium will be administered in blind fashion except that a case "coordinator" will be unblind. The first lithium administration will begin with the mean child dose for that age and will be adjusted until a blood level between .8 and 1.2 mg/liter is obtained. The case coordinator will adjust the number of tablets for a matched placebo case in parallel with adjustment for active drug.

Side effects will be monitored. The second period of lithium administration will begin with that child's effective dose.

1) The patients will be seen on a weekly basis for evaluation and administration of medication. At each visit the following procedures will be carried out: A brief structured interview of 15-30 minutes with a special focus on the target symptoms. The interview will be rated on the target items of the CPRS and the CARS. Lithium levels will be obtained in serum. A global rating scale will be filled out by the interviewer on each diagnosis made. 2) The parents will rate their target symptoms weekly and a global rating will be done by the parent interviewer also.

The AER will be repeated on a schedule regulated by the unblind lithium administration.

Major Findings: To date, a total of 6 patients have completed the study and 5 more are being evaluated. Table II gives the children's diagnoses and the children's response to lithium, as rated independently by the child's interviewer, the parent's interviewer and the teacher.

Two girls, (A & B) ages 8 and 12, diagnosed as having a bipolar affective disorder, mixed, responded to lithium on both the child as well as parental ratings. B had a dramatic response to lithium in both manic and depressive symptoms, but the mother kept occasionally giving lithium during placebo periods (verified by positive lithium blood levels), making statistical evaluation impossible. A boy (C) age 11, diagnosed as having an attention deficit disorder with hyperactivity and overanxious disorder was rated as lithium responsive on the basis of the parents' reports, but not on the reports of the child's interviewer. The teacher's rating closely approached significance. An 11 year old boy (D) with three diagnoses: Major depressive disorder, recurrent, attention deficit disorder with hyperactivity and overanxious disorder was rated as lithium responsive by the child's rater only on overanxious disorder, but not on the other diagnoses. The parent's and teacher's ratings were negative for all diagnoses. The two remaining children showed no response to lithium on any rating: (E) a boy age 12 with an attention deficit disorder with hyperactivity and cyclothymic disorder, (F) a boy aged 10 with encopresis and major depressive disorder, recurrent.

EP Results

The full set of EPs was obtained on 5 children; one child (B) received only the baseline and 2 lithium sessions. For the purposes of predicting lithium response we used the average of the P100 amplitude/intensity slope for the two placebo sessions in 5 children and the baseline in the sixth (B).

The evaluation of the efficacy of the EP augmenting/reducing measure in predicting lithium response cannot be statistically confirmed in this small sample. However, it is of interest that the strongest augmenter, patient A, was the only child to show both significant global child and global parent rating improvement. The two patients with significant global parent rating improvement also had higher amplitude/intensity slopes than the three with nonsignificant rating change (Table II). It is interesting that patient B who clinically appeared to respond dramatically was the second highest augmenter.

EP changes with lithium were similar to those observed with lithium in adults - a significant increase in amplitude for the N120 component, from placebo to lithium, occipital lead (Oz) for all 5 children. The average amplitude (across all intensities) went from 9.09 μv to 13.83 μv ($F=18.08$, 1, 4, n.f., $p<0.01$). The P100 component at Oz also showed a significant enhancement ($F = 10.20$, $p < 0.05$). This pattern was also maintained in patient B, 12.91 μv baseline, 13.69 μv on lithium.
In conclusion, even our small sample gives further credence to the efficacy of lithium in certain childhood disorders. Our best 2 responders not only had a BP diagnosis but an electrophysiological pattern, augmenting, which has previously been associated with lithium response. This, taken together with the similarity of the EP changes on lithium to those occurring in adult patients treated with lithium, supports a physiological parallel between bipolar affective illness in adults and children.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00151-04 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Behavior and Electrophysiological Response of Normal and Hyperactive Children and Normal Adults to a Single Dose of Dextroamphetamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Theodore P. Zahn, M.D.	Research Psychologist	LP	NIMH
	Monte S. Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BP	NIMH
	Christy L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS
	Herbert Weingartner, Ph.D.	Acting Chief	LP	NIMH
	Michael H. Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH
	Irwin J. Kopin, M.D.	Chief	LCS	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, DCBR, NIMH; Section on Experimental Therapeutics, LCS, DCBR, NIMH; Communicative Disorders Program, NINCDS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 00	PROFESSIONAL: 00	OTHER: 00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project has been discontinued

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00152-03 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Sinemet on Behavior in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Gerald L. Brown, M.D.	Medical Officer	BP	NIMH
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	Dennis H. Langer, M.D.	Clinical Associate	BP	NIMH
	Irwin J. Kopin, M.D.	Chief	LCS	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH
	C. Raymond Lake, M.D.	Associate Professor	DPP	USUHS

COOPERATING UNITS (if any)

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Laboratory of Clinical Science, DCBR, NIMH, Department of Psychiatry & Pharmacology, USUHS

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A double-blind crossover study evaluated the clinical response of hyperactive boys to Sinemet (maximum dose, 60-600 mg). The behavioral and cognitive responses were qualitatively similar to that seen with amphetamine but the effects were much weaker; clinically it does not appear the Sinemet will be useful in the treatment of hyperkinetic children. Theoretically, Sinemet increased urinary MHPG, an index of central norepinephrine metabolism and therefore the mild improvement seen does not support a specific dopamine hypothesis of therapeutic action of stimulants in these children.

Project Description:

Objectives: Hyperactive children usually respond symptomatically to stimulant medications which are known to increase the concentration of monoamines at the receptor sites in the brain. As L-dopa is known to increase the availability of dopamine in the brain, if a deficiency of dopamine were responsible for at least some cases of hyperkinesis, then L-dopa should be expected to have some therapeutic value.

Methods Employed: The recent practice of combining L-dopa and Carbidopa, a peripheral dopa decarboxylase inhibitor, lowers the necessary dosage and thereby decreases side effects, making this treatment more feasible for children. Hyperactive boys, ages 6-12, exhibiting restlessness and impulsivity both at school and during an inpatient evaluation period were selected for the protocol. Evaluation included medical, neurological, and psychiatric examination as well as psychological testing.

Children were started on a gradually increasing dosage of Sinemet or placebo; medication was increased over the first two weeks to the maximum dose (60-600 mg Sinemet or 6 tablets of placebo), which was given during the third week as tolerated.

During the third week of placebo or Sinemet treatment, plasma norepinephrine and HVA turnover was assessed. Clinical response for the group was compared for drug and placebo periods and individual responses were related to changes in the catecholamine measures.

Major Findings: The project began in June 1978. Eight children received the drug. Because of nausea and vomiting, two children received a maximum dose of 40/400 mg/day of Carbidopa/L-dopa. For the group of eight children completing the study, Sinemet causes a small but significant decrease in hyperactivity on the teacher rating scale. There was a slight decrease in motor activity but this was not significant. There was a trend for improvement on the continuous performance test, a measure of vigilance. There was no improvement on cognitive test performance, in contrast to the highly significant effects seen with amphetamine in similar populations. Plasma NE measured in Dr. Lake's laboratory did not change significantly; urinary MHPG and urinary VMA on the other hand both were significantly elevated. There was no significant correlation between individual improvement scores and changes in urinary MHPG or VMA.

Significance to Mental Health Research: As hyperactivity may be a forerunner of adult sociopathy, alcoholism or schizophrenia, studies on treatment and underlying pathophysiology of hyperactive children have wide application throughout the field of mental health research. Alternate treatments to stimulants are practically useful, as approximately 15% of hyperactive children do not respond favorably to stimulants.

Proposed course of Project: The project was terminated after eight children completed the study. While there was some statistical improvement in behavior, it was clear the Sinemet was an inferior treatment to that seen with stimulants.

Publications: Langer, D., Rapoport, J., Brown, G., Ebert, M., Bunney, W.E., Jr.: Behavioral Effects of Sinemet in Hyperactive Boys. Submitted for Publication. J. Amer. Acad. Child Psychiatry.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00153-03 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Treatment of Obsessional Children and Adolescents with Chlorimipramine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Robert N. Elkins, M.D.	Clinical Associate	BP	NIMH
	Leon Cytryn, M.D.	Medical Officer	BP	NIMH
	Dennis L. Murphy, M.D.	Chief	CN	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch, DCBR, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.10	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obsessional disorder of childhood is a rare disorder about which little is known. The purpose of this protocol is to collect basic data on family history of mental illness, sleep measures and neuropsychological testing on a selected group of children and adolescents with obsessional disorder. A drug trial of chlorimipramine, desmethylimipramine, and placebo will be carried out to evaluate the effect of antidepressant and the specificity of chlorimipramine for this disorder.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To evaluate the clinical response of Obsessive-Compulsive disorder of childhood to chlorimipramine and to relate this response to changes in ratings of depression. Clinical response to chlorimipramine will be related to platelet serotonin at baseline.

Methods Employed: Patients are sought on a national level because of the rarity of the disorder. Inclusion criteria are Obsessive-Compulsive disorder as a primary disturbance. Children must have IQ of 85 or above, and be free from known neurological disturbance or psychosis.

A modification of the Leyton Obsessional Inventory will be used to monitor drug effects on Obsessive-Compulsive symptomatology throughout the 16-week clinical trial. Weekly ratings are made by two physicians and ward nurses on the CPRS.

Plasma levels of drug as well as platelet serotonin will be assayed for each drug phase.

Major Findings: Nine children have entered the protocol: six males aged 13-15, and two females aged 13 and 15. The first patient improved gradually throughout his hospital course and did not have a different course during chlorimipramine treatment. Depression ratings changed in parallel with obsessive symptoms. One patient showed dramatic improvement on chlorimipramine and is being maintained on the drug. The others have showed equivocal response.

A problem in interpreting drug effects is the tendency for there to be gradual improvement over the course of the study for almost all of the patients. This group trend obscures individual drug/placebo differences and may mask true drug effects.

Preliminary analysis of family pedigree data is somewhat at variance with other reports of obsessive-compulsive disorders. Only one patient reports obsessive-phobic symptomatology in any family members. The other families have no obsessional illness among first degree relatives. There were a surprising number of siblings who exhibit learning and behavior problems. Six of the 17 siblings age 18 and under have such symptoms. Sleep recordings on subject No. 1 showed marked decrease in REM latency at baseline, which increased over time with clinical improvement. These findings indicate a possible biologic link between depression and obsessive-compulsive disorder in childhood. Speech and language testing suggests mild dysphasia suggestive of a dysfunction of frontal and/or temporal lobes.

Platelet MAO and 5HT from the patients and a group of matched adolescent controls do not appear different. Plasma tricyclic levels are being assayed but are not yet available.

Significance to Mental Health Research: Obsessive-Compulsive Disorder is a rare but extremely disabling condition. About one third of adults with the disorder had their onset during childhood or adolescence. Children with the condition are often very ill and do not respond well to treatment. Relative to other rare conditions such as infantile autism, there has been virtually no research in this area of childhood mental illness.

Proposed Course of Project: A total of 20 patients will be studied. Baseline clinical measures, plasma tricyclic level, and platelet serotonin will be examined in relation to clinical response to the active drugs.

Publications:

Elkins, R., Rapoport, J., and Lipsky, A.: Obsessive-Compulsive disorder of children and adolescents. *J. Am. Acad. Child Psychiatry* (In Press).

Rapoport, J., Elkins, R., and Lipsky, A.: A Pilot Trial of Chlorimipramine for Adolescents with Obsessive-Compulsive Disorder. *Psychopharmacology Bulletin,* In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00154-02 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

State Dependent Learning in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Dennis H. Langer, M.D.	Clinical Associate	BP	NIMH
	Herbert Weingartner, Ph.D.	Acting Chief	LP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .40	PROFESSIONAL: .10	OTHER: .30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

State dependent learning has primarily been demonstrated in adults receiving substances which impair learning. However, two studies have raised the possibility that hyperactive children whc often learn material on stimulant drug may manifest state dependency as measured by decreased transfer of learning to the off-drug state.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Hyperactive boys who also are at least one year behind age/IQ norms in academic achievement will be tested on and off dextroamphetamine (0.5 mg/kg) to see whether verbal material is best retained in the same or different state from which it is learned.

Methods Employed: Twenty hyperactive boys will be tested in four paired learning and retention sessions. Learning and recall will be measured during same (drug-drug or placebo-placebo) sessions and different (placebo-drug or drug-placebo) sessions. All sessions will be carried out using a double blind, random assignment, counterbalanced order design. Mood scale ratings will also be completed for each child for each test session.

Major Findings: A total of fifteen children have been studied to date. Preliminary analysis of the data indicate some support for existence of state dependent learning.

Amphetamine state dependent learning (SDL) is evident in an asymmetrical manner in many, but by no means all, children tested under four different learning-remembering conditions. SDL, when present, is more evident for poorly processed information (as opposed to well-learned information). SDL-related memory dissociations, when they do appear in stimulant-treated children, are seen on tests of free recall as well as recognition memory. In general, the appearance of amphetamine SDL in children is less reliable and less complete than dissociations that appear following treatment with CNS depressants in adults.

Significance to Mental Health Research : A major clinical problem in the treatment of hyperactive children is the lack of improvement in academic performance on stimulants in contrast to the clear change in impulsive inattentive behavior. If learning on stimulants were to a significant extent state dependent, this would shed light on an important and to date puzzling area.

Proposed Course of Project: The study will be completed by July 1980. If state dependency is not demonstrated then the study will terminate. If state dependency is found then we will evaluate state dependency in children receiving chronic amphetamine treatment as this is more applicable to clinical use.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00161-02 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cognitive Effects of Dietary Substances in Normal and Learning Disabled Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
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	N. Sitaram, M.D.	Staff Psychiatrist	BP	NIMH
	Alan Neims, Ph.D.	Professor of Pharmacology of Pediatrics, U. of Flordia, Gainesville, Florida		

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, DCBR, NIMH;
Section on Experimental Therapeutics, LCS, DCBR, NIMH; U. of Fla., Gainesville

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.30	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Both caffeine and lecithin, substances within the diet of normal children, have been shown to affect memory and vigilance of normal adults. The effects of these substances will be studied on normal children. Both acute and subacute effects of caffeine will be studied.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To evaluate the effects of caffeine (4 and 10 mg/kg) in single doses and lecithin (60 gm/day for up to 5 days) on memory and vigilance of normal children.

Methods Employed: Children will be paid as normal volunteers for participating in this study. Children must be free from psychiatric disorder or cognitive disability.

Double-blind single dose trials of caffeine or lecithin will be carried out, using cognitive test response as the dependent measures. Side effects will also be evaluated.

One 9 year old boy with learning disabilities was studied on lecithin (60 mg/day) or placebo using a single dose design. This child was selected as the parents had claimed that supplementary dietary lecithin had improved the childs grades and behavior.

Double-blind studies of single doses of caffeine (3mg/kg and 10 mg/kg) were conducted with 19 grade school boys. In addition, there is an ongoing study of subacute effects of caffeine (10 mg/kg day). In this latter study, subjects had daily caffeine or placebo for two week periods in a double-blind crossover design.

Major Findings: Lecithin had no effect on the individual child tested. Further work was not carried out as parallel studies with adult volunteers and senile patients showed lecithin effects to be minimal.

Acute caffeine study: Single doses of caffeine (10 mg/kg) had several significant effects on the normal children's cognitive and behavioral responses. In contrast to placebo or the lower dose (3 mg/kg), high doses of caffeine increased motor restlessness, while improving vigilance as measured by reaction time and continuous performance test.

The subacute caffeine study is presently ongoing. Preliminary data however indicate that during the caffeine period, parents and teachers rate the children as more restless, more nervous and jittery. These findings have implications for the biological basis of anxiety.

Significance to Mental Health Research: There is little known about the influence of diet on normal children's behavior. Caffeine is widespread in our daily diet and the influence of dietary level on both normal and pathological samples has considerable clinical significance. Theoretically, the effects of caffeine resemble mild clinical anxiety. Caffeine may provide a pharmacological model for anxiety in childhood.

Proposed Course of Project: The subacute study will be completed during 1980-81. During this time a comparison group of adults who receive either placebo, 3 or 10 mg/kg of caffeine will be studied on identical measures to that of children. The point of the latter study is to see whether children are differentially sensitive to caffeine effects as has been suggested.

Finally, examination of recent survey data will be carried out to relate current dietary intake in comparable populations to the findings from these studies. We are also considering having a subgroup of caffeine sensitive children return for a study of whether benzodiazepine blocks the caffeine effect.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00162-01 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Treatment of Hyperactive Children with Desmethylimipramine or Mianserin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
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	William Potter, M.D., Ph.D.,	Assistant to Chief	CP	NIMH
	Michael Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH

COOPERATING UNITS (if any)

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Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mianserin, a presynaptic alpha blocker, has been reported to be an effective antidepressant in adult depressed patients. A trial of Mianserin and desmethylimipramine is planned to compare the acute and chronic efficacy of these agents in hyperactive children and to relate clinical effects to measures of norepinephrine metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: As tricyclic antidepressants have been shown to have short term beneficial effects for hyperactive children, this study is designed to examine these effects more closely. Mianserin, a presynaptic alpha blocker, is thought to exert clinical efficacy in depression by producing a larger amount of functional norepinephrine post synaptically by a feedback mechanism. DMI acts, in contrast, primarily by blocking reuptake of norepinephrine. By monitoring plasma NE, urinary MHPG and examining clinical effects, changes in NE metabolism and antihyperactive effects could be examined to see if they are related.

Methods Employed: Mianserin was tried in an open pilot study in 5 hyperactive grade school children attending the 2-West day program. A comparison group of 5 children who were given placebo over a three week period was also examined on the same measures. Measures included: Vigilance (CPT), Teacher Rating Scale, Cognitive Testing and Side Effects. Maximum dose of Mianserin was 60 mg/day.

Major Findings: Mianserin did not appear helpful clinically in any of the five children in this pilot study. Compared with the placebo contrast group, there was no change in any behavioral or cognitive measure and this agreed with the general consensus of ward and educational staff. On the other hand, there seems to be postural hypotension which occurred in two children and had a clinically significant effect. This is not reported with adult patients or volunteers.

Significance for Mental Health Research: Hyperactivity is a major issue in child psychiatry. Understanding its mechanism may lead to more effective treatment and prevention.

Projected Course of Project: As Mianserin does not appear clinically useful, the protocol will be continued using DMI and placebo in a double-blind noncrossover design. Clinical efficacy of DMI will be examined in relation to chronic (1 month) drug and chemical effects will be compared and related to clinical effects. In addition, plasma tricyclic level, platelet tricyclic recepton binding, and pupillary sensitivity to phenylephrine and hydroxyamphetamine will be studied. The latter measures pre- and post-synaptic alpha-adrenergic function.

Publication:

Rapoport, J., Langer, D., Ebert, M.: Pilot Trial of Mianserin Treatment of hyperactive Boys. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00163-01 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Naturalistic Study of Activity Levels of Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	Linda Porrino, Ph.D.	Guest Worker Unit on Childhood Mental Illness	BP	NIMH
Other:	Judith Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
	Thomas Wehr, M.D.	Chief, Clinical Research Unit	CP	NIMH

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .50	PROFESSIONAL: .40	OTHER: .10

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Because of the new technology for measuring 24 hour activity using the NIH actometor, a study has been designed to examine activity levels of hyperactive children and matched controls at baseline. Following this, activity of hyperactive group will be compared during amphetamine and placebo treatment periods. This is the first study to examine drug effects on motor activity outside of a laboratory setting. Drug effects will be examined in relation to measures of structure of classroom and home environments.

Project Description:

Objectives: Hyperactive boys and matched controls who are in the same school, grade and classes will be followed for a baseline week to compare 24 hour activity patterns for the groups. Following this, hyperactive boys will be monitored continuously for four weeks. The effects of amphetamine (15 mg) or placebo will be compared in a double-blind, ABAB design.

Methods Employed: A total of 20 hyperactive-control pairs will be studied. Activity levels during school, free play and home activities will be compared and related to measures of attention (Continuous Performance Test), and ratings of school and home structure. Children will wear the monitor on belts even when they sleep. Weekly appointments will be kept at which time parent and teacher behavior ratings, side effect, hourly diaries of weekly activities and attentional measures will be obtained.

Major Findings: A total of 3 patient-control pairs have been started. Preliminary findings indicate that hyperactive children are significantly more restless than controls although not markedly so. Drug effects appear biphasic, decreasing activity during the day with some increase in activity in the evening. Whether this represents a "rebound" effect, or simply a delayed effect of insomnia secondary to continued direct effect of the drug is not clear.

Significance to Mental Health Research: Only a naturalistic study can relate laboratory findings to clinically relevant situations. The nature of "hyperactivity" is poorly understood. If hyperactive children are truly more restless than their peers it is important to know for what situations this is true. Furthermore, this study will provide data on behavioral "rebound" in the evenings following drug. If this is a major finding, then altered dose schedule would be indicated.

Proposed Course of Project: A total of 20 hyperactive children and their matched controls will be studied. In addition, structured interview of these outpatients in comparison with interviews of "hyperactive" children attending the 2 West day program will be compared to explore the many different diagnostic categories included in the heterogeneous population of "hyperactive" children. Activity patterns from the naturalistic study will be related to diagnostic subgroup.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00164-01 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

A Study of Infants of Parents with Bipolar Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Cytryn, M.D.	Medical Officer	BP	NIMH
Other:	Yolande Davenport, M.S.W.	Social Worker	LDP	NIMH
	Martine Lamour, M.D.	Guest Worker	BP	NIMH
	Donald H. McKnew, M.D.	Medical Officer	BP	NIMH
	Marian Yarrow, Ph.D.	Research Psychol.	LDP	NIMH
	Carolyn Waxler, Ph.D.	Research Psychol.	LDP	NIMH
	Ann Barnett, M.D.	Children's Hospital	Washington, D.C.	
	Ira Weiss, Ph.D.	Children's Hospital	Washington, D.C.	
	Robert Harmon, M.D.	Univ. of Colorado	Denver, Col.	
	Theodore J. Gaensbauer, M.D.	Univ. of Colorado	Denver, Col.	
	William E. Bunney, Jr. M.D.	Chief	BP	NIMH
	Frederick Goodwin, M.D.	Chief, Clinical Psychobiology	CPB	NIMH

COOPERATING UNITS (if any)

LDP, NIMH, University of Colorado, Denver, Colorado; Children's Hospital, Washington, D.C.; Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.4	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this study is to investigate 1) affective states, neuropsychobiological patterns and general development of infants with a parent with bipolar illness; 2) To study the infant-family interaction, particularly the difference between the ill and healthy parent. There is general agreement about the genetic vulnerability of first degree relatives of manic-depressive patients. Such genetic vulnerability may possibly be already evident during infancy. The same may be said about environmental factors, especially the family, causing an affective disorder, which too, may be identified and detected by the study of infant-family interactions.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The major purpose of this study is to investigate 1) affective states, neuropsychobiological patterns and general development of infants with a parent with bipolar illness; 2) To study the infant-family interaction, particularly the difference between the ill and healthy parent. There is general agreement about the genetic vulnerability of first degree relatives of manic-depressive patients. Such genetic vulnerability may possibly be already evident during infancy. The same may be said about environmental factors, especially the family, causing an affective disorder, which too, may be identified and detected by the study of infant-family interactions.

Methods Employed: The development of infants having one parent with a bipolar affective disorder was studied. Two groups were investigated 1) 6 male infants with one parent who met the RDC criteria for bipolar I illness (3 fathers, and 3 mothers), 2) 6 matched controls, each of whom has both parents free of any psychopathology. Each infant was studied 3 times, at 12-13 months, at 15-16 months, and at 18-19 months. The studies included neurological examination, averaged evoked responses (AER), measurement of affect, Bayley Developmental Scale, temperamental profile, parental rearing attitudes and behaviors, and a detailed family history with emphasis on family psychodynamics.

Major Findings: All the data collection has been completed. Each index and control infant had the following: Neurological examination, developmental examination, average evoked potential, temperamental assessment and three stranger-separation sessions. In addition detailed family assessment and history was obtained. The data are being evaluated. It is anticipated that this evaluation will be completed by late summer or early fall, 1980.

Significance to Biomedical Research and the Program of the Institute: This study may find early behavioral markers of vulnerability to manic-depressive illness. It is, along with the research in the Child Unit on children at risk for later psychiatric disorder, an important area for preventive psychiatry.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00034-10 BP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Psychological and Physiological Correlates of the Average Evoked Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Sect. on Clin. Psychophysiology	BP NIMH
OTHER:	R. Coppola, D.Sc.	Electrical Engineer Sect. on Clinical Psychophysiology	BP NIMH
	R. A. Lavine	George Washington Univ. Washington, D.C.	Geo. Wash. Univ.

COOPERATING UNITS (if any)

George Washington Univ., Washington, D.C.

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Evoked potentials at varying intensities of stimulation were examined in normal volunteers under different conditions of attention. A positive wave at 30 msec recorded over the post-central gyrus was found to be enhanced when subjects attended to the stimulus -- the earliest component yet found to be associated with attentional enhancement.

PHS-6040
(Rev. 10-76)

Project Description:

Evoked potential research has been given emphasis by the Section because evoked potentials show high heritability and sensitivity to attentional as well as neuropharmacological manipulation -- coupled with the possibility of cortical or subcortical localization, these features make it an attractive technique for biological research in psychiatry.

Previous studies in man have reported increases in the amplitude of late secondary evoked potential components with attention, but not primary components. In this study, somatosensory evoked potentials were recorded over somatosensory cortex in normal volunteers while they were counting stimuli and while they were doing mental arithmetic.

For peak P30 recorded over the post central gyrus the amplitude for the attention condition was significantly greater ($p<.01$) than the amplitude for the distraction condition. This occurs whether the peak is measured relative to the mean of the entire 512 msec recording epoch or relative to a local (5-75 msec) baseline. Using the latter baseline, 11/14 subjects showed attentional enhancement of amplitude. A peak-to-peak measure, P30 - N70, also showed a significant difference ($p<.02$) in the same direction. Thus P30 was increased during the attention condition whether measured from either baseline or from the succeeding N70 peak. No significant differences between conditions for peaks P15, P50, or N70 were found. Nor were significant differences in peak latency found.

Our results indicate that attentional processes are capable of modulating incoming somatosensory information as early as the first cortical synapses. Whether our P30 changes represent orientation of the CNS toward processing external stimuli suppression of response to external stimuli during internal tasks (e.g. mental arithmetic) or the first stage of selective attention remains to be resolved with further multitask comparisons.

Publications:

Nurnberger, J.I., Gershon, E.S., Murphy, D.L., Buchsbaum, M.S., Goodwin, F.K., Post, R.M., Lake, C.R., Guroff, J.J., and McGinniss, M.H.: Biological and clinical predictors of lithium response in depression. In Gershon, S., Kline, N.S., and Schou, M. (Eds.): International Lithium Conference: Controversies and Unresolved Issues. New Jersey, Excerpta Medica, ICS Series, 1979, pp. 241-256.

Buchsbaum, M.S.: Electrical activity of the brain: Age changes and personality differences, in: Proceedings of Council Seminars, Duke University, Center for the Study of Aging and Human Development, Duke University Medical School, Durham, N.C., (In Press).

Lavine, R. A., Buchsbaum, M. S., and Schechter, G. Human Somatosensory evoked responses: Effects of attention and distraction on early components. Physiological Psychology. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00035-08 BP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical and Psychopharmacological Correlates of the Average Evoked Response in Psychiatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:

M.S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology,	BP,NIMH

Other:

F.K. Goodwin, M.D.	Chief,	CP,NIMH
D.L. Murphy, M.D.	Chief,	CN,NIMH
T.P. Zahn, Ph.D.	Research Psychologist	LPP,NIMH

(R.D. Coursey, M.D., University of Maryland; R.J. Haier, M.D., Brown University; I.I. Gottesman, M.D., University of Minnesota)

R.J. Wyatt, M.D.	Chief	LCP,NIMH
J.E. Kleinman, M.D.		LCP,NIMH

COOPERATING UNITS (if any) Clinical Psychobiology Branch; Clinical Neuropharmacology Branch; Laboratory of Psychology and Psychopathology; DCBR, NIMH. Department of Clinical Psychology, University of Maryland; Department of Psychiatry, University of Kentucky; Department of Psychiatry, Brown University, Providence, Rhode Island;

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.6	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A population of individuals potentially at risk for psychiatric disorders was identified by screening 375 college student volunteers for low platelet monoamine oxidase (MAO) activity levels. The lower and upper 10 percent in MAO activity were interviewed and average evoked response, personality, perceptual and family history data obtained. Low MAO probands reported more frequent psychiatric or psychological counseling and problems with the law. Families of low MAO probands had an eight-fold increase in suicide incidence over those of high MAO probands. Both in these low MAO probands and in NIH psychiatric inpatients, the combination of low MAO and average evoked potential augmenting was associated with increased frequency of suicide or suicide attempts. This suggests that reduced MAO levels, reported previously in patients with affective disorders and chronic schizophrenia, may predict a vulnerability to psychiatric disorder. This year we have followed up the high risk individuals 2 years after identification, studied their relatives directly with both biochemical measures and interviews and reviewed the relationship of MAO to psychological traits in review articles. A NIMH conference sponsponsored by the Center for studies of schizophrenia was organized on MAO and an issue of the Schizophrenia Bulletin was devoted to the proceedings.

Cooperating Units (Continued)

and Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota.

Project Description

We previously found that the combined multivariate use of platelet MAO level and AER allowed us to successfully separate the four groups; normal, schizophrenic, bipolar, and unipolar affective illness, with 72% accuracy. However, since hospitalization stress, sensory isolation, dietary changes, etc., might affect both MAO and AER, we planned to locate normal individuals with extreme values of MAO and to study them with AER measures. This then represented a kind of biochemical "high-risk" study for the major psychoses.

Using a biological high risk paradigm, the "at risk" subjects were chosen from 375 college students on the basis of extremely low blood platelet monoamine oxidase (MAO) activity levels. The sample was comprised of 19 low and 17 high MAO males and 17 low and 15 high females. These groups were further subdivided on the basis of their augmenting/reducing evoked potentials. Psychological tests assessing dimensions relevant to schizophrenia and affective illness were administered and the scores were factor analyzed. As expected, the "at risk" group of low MAO males scored significantly higher on a general psychopathology factor. Moreover, the subgroup of males who theoretically would be at risk for schizophrenia (low MAO with reducing evoked potentials) gave significantly more remote word associations than the high MAO augmenters. This interaction effect of MAO and evoked potentials was also significant for females. Significant sex differences were found on the mania factor (the males were more active) and remote associations factor (males gave more remote associations). In addition, a significant interaction effect of sex and MAO on the general psychopathology factor was found with the low MAO males and high MAO females scoring in the more pathological direction.

Thirty-seven parents of probands with low platelet monoamine oxidase (MAO) activity levels and 38 parents of high MAO probands were examined for MAO activity, past and present psychopathology, and reported psychopathology in other relatives. Results showed generally positive and significant correlations between parents' and children's MAO levels, significantly greater rates of "high MAO related" disorders in parents and relatives of high MAO probands and of "low MAO related" disorders and symptoms in parents and relatives of low MAO probands. Support for a two-directional monoamine hypothesis of affective disorders is suggested.

Chronic alcoholic patients were also studied in collaboration with Dr. A. Ludwig, Chairman, Department of Psychiatry, University of Kentucky. It is hypothesized that alcoholics possess a perceptual neurophysiological sensitivity which renders them vulnerable to the effects of information or stimulus overload; the resort to alcohol, therefore, may represent an adaptive attempt to restore physiologic homeostasis through the pharmacologic "dampening" properties of this drug. Twelve alcoholic and 12 normal subjects were assigned to three different levels of audio-visual sensory stimulation (no, medium, high) and three different doses of alcohol (placebo, low, high) according to a balanced incomplete block design. Autonomic arousal was assessed during the conditions.

Essentially, the results indicate no significant effects for sensory conditions or sensory ethanol interactions within groups for any of the dependent variables. There are significant increases in heart rate for both groups associated with increased levels of ethanol. Most of the significant differences noted pertain to between-group differences. In comparison to controls, alcoholics demonstrate elevated craving, mood dysphoria, mean heart rate, and reaction time across all sessions and independent of condition.

Aside from the issue of group and interactional differences, the results more clearly reveal the impact of sensory stimulation on physiological parameters. For the most part, as levels of sensory stimulation increased, both alcoholics and social drinkers displayed significant increases in mean heart rate and EEG frequency, both variables representing traditional indices of "arousal."

While the results of this study are inconclusive, when taken in the context of other findings demonstrating potential differences in sensory processing among alcoholics, they do indicate the need for further investigation in this intriguing area. Further research will perhaps compensate for potential methodological problems in the current study, such as the small number of subjects and the postwithdrawal status of alcoholics.

A prospective investigation using platelet monoamine oxidase activity and cortical evoked response augmenting/reducing to predict the onset of new episodes of affective disorders was conducted in a college sample. This sample was selected by screening 385 male college student volunteers on the Minnesota Multiphasic Personality Inventory (MMPI). A group of students with highly aberrant MMPI profiles was identified by selecting the 34 of the 385 students who had at least one original MMPI scale T score of 90 or above (over 4 standard deviations above the original population mean on which the MMPI was standardized). A comparison group of 27 students with normal profiles also was selected. These 61 subjects completed MAO and evoked potential determinations as well as psychiatric interviews conducted using the Schedule for Affective Disorders and Schizophrenia-life history version (SADS-L) and the RDC. The purpose of screening on the MMPI was to identify subjects with serious psychological problems so that the subsequent biological classification could be evaluated with as many deviant cases as possible. Together, these 61 students represent a group having a very heterogeneous psychological status (ranging from normal with no history of problems to individuals with RDC-defined major depression, bipolar II episodes, antisocial personalities and serious drug abuse). During an 18-month period between clinical interviews, higher incidences of major depression and hypomania characterized the low MAO/augmenting and the high MAO/reducing subjects. These same subgroups also had been associated with affective psychopathology in the original retrospective study.

We reviewed the trait of sensation seeking and the biological phenomena associated with it: strength of the initial orienting reflex, augmenting versus reducing of the average evoked potential, the enzyme monoamine oxidase, and gonadal hormones (both androgen and estrogens). Both the psychological test and biological measures have retest reliabilities that are characteristic of stable traits. Twin studies show moderate to strong genetic control of the personality and of several of the biological measures. Several behavioral characteristics that are associated with the trait in humans are also related to the biological traits in animals, including sociability and social dominance, reactivity to novel situations, and play. In humans, sexual experience, interest in new situations, experiences with drugs, manic-depressive tendencies, and psychopathy are associated with both sensation seeking and its biological correlates. Age and sex differences show some parallels and some differences between sensation seeking and its biological correlates. The convergence of findings points up some new directions for research in the role of central monoamines and their regulators in the development of basic personality traits.

Lastly, the normal volunteers with low MAO were useful in demonstrating a relationship between platelet MAO and brain monoaminergic activity. In these normal volunteers with low monoamine oxidase activity and in unmedicated patients with chronic schizophrenia, plasma prolactin concentrations varied directly with platelet monoamine oxidase activity. No such relationship was found in normal subjects with high platelet monoamine oxidase activity. These data suggest that platelet monoamine oxidase activity reflects monoaminergic activity in the tubero-infundibular system, which in turn affects plasma prolactin concentrations. This relationshp may be important in patients with low platelet monoamine oxidase activity, such as some chronic schizophrenics.

Publications

Buchsbaum, M.S., Murphy, D.L., Coursey, R.D., Lake, C.R. and Zeigler, M.G.: Platelet monoamine oxidase, plasma dopamine-beta-hydroxylase and attention in a "biochemical high risk" sample. J. Psychiatr. Res. 14: 215-224, 1978.

Coursey, R.D., Buchsbaum, M.S. and Murphy, D.L.: Platelet MAO activity and evoked potentials in the identification of subjects biologically at risk for psychiatric disorders. Br. J. Psychiatry 134: 372-381, 1979.

Lipper, S., Murphy, D.L., Slater, S. and Buchsbaum, M.S.: Comparative behavioral effects of clorgyline and pargyline in man: A preliminary evaluation. Psychopharmacology 62: 123-128, 1979.

Ludwig, A.M., Wikler, A., Buchsbaum, M.S., Cain, R.B. and Vannier, M.W.: Similarity of effects of alcohol and sensory stimulation in post-alcoholics and controls. Alcoholism (N.Y.) 3: 248-251, 1979.

Ludwig, A.M., Wikler, A., Buchsbaum, M.S., Cain, R.B. and Vannier, M.W.: Physiological responses of men alcoholics and controls to varying sensory stimulation. J. Stud. Alcohol 40: 472-475, 1979.

Murphy, D.L., Lipper, S., Campbell, I.C., Major, L.F., Slater, S.L. and Buchsbaum, M.S.: Comparative studies of MAO-A and MAO-B inhibitors in man. In Singer, T.P., VonKorff, R.W. and Murphy, D.L. (Eds.): Monoamine Oxidase:

Structure, Function, and Altered Functions. New York, Academic Press, 1979, pp. 457-475.

Donnelly, E.F., Murphy, D.L., Waldman, I.N., Buchsbaum, M.S. and Coursey, R.D.: Psychological characteristics corresponding to low versus high platelet monoamine oxidase activity. Biol. Psychiatry 14: 375-383, 1979.

Kleinman, J.E., Pitkin, S., Rogol, A., Buchsbaum, M.S., Murphy, D.L., Gillin, J.C., Nasrallah, H.A. and Wyatt, R.J.: A correlation between platelet monoamine oxidase activity and plasma prolactin concentrations in man. Science 206: 479-481, 1979.

Haier, R.J., Buchsbaum, M.S., Murphy, D.L., Gottesman, I.I. and Coursey, R.D.: Psychiatric vulnerability, monoamine oxidase, and the average evoked potential. Arch. Gen. Psychiatry 37: 340-345, 1980.

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Haier, R.J., Buchsbaum, M.S. and Murphy, D.L.: An 18-month follow-up of students biologically at risk for psychiatric problems. Schizophr. Bull. 6: 334-337, 1980.

Puchall, L.B, Coursey, R.D., Buchsbaum, M.S. and Murphy, D.L.: Parents of high risk subjects defined by levels of monoamine oxidase activity. Schizophr. Bull. 6: 338-346, 1980.

Buchsbaum, M.S. and Ludwig, A.M.: Effects of sensory input and alcohol administration on visual evoked potentials in normal subjects and alcoholics. In Begleiter, H. (Ed.): Biological Effects of Alcohol. New York, Plenum, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00036-06 BP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Individual Differences in Sleep and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Sect. on Clin. Psychophysiology	BP	NIMH
OTHER:	J. C. Gillin, M.D.	Research Psychiatrist	BP	NIMH
	R. M. Post, M.D.	Chief, Section on Psychobiology	BP	NIMH
	W. B. Mendelson, M.D.	Research Psychiatrist	SMR	NIMH

COOPERATING UNITS (if any)

Clinical Psychopharmacology, St. Elizabeths Hospital, Washington, D. C.

LAB/BRANCH Biological Psychiatry Branch

SECTION Clinical Psychophysiology

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New techniques for analysing local EEG processes during sleep stages are under development.

PHS-6040
(Rev. 10-76)

Project Description:

Sleep EEGs were obtained on three college students using 14 channels of EEG all placed on the left hemisphere. Ten-second epochs from each sleep stage were analysed spectrally using FFT techniques. Programs to create maps of EEG activity as interpolated, artificial color scales are under development. Coordination with 18F-2DG studies is planned.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00037-08 BP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Genetic Factors in Psychiatric Illness and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:

M.S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP,NIMH

Other:

E.S. Gershon, M.D.	Chief, Section on Psychogenetics	BP,NIMH
R. Cromwell, M.D.	University of Rochester	
R.D. Coursey, M.D.	University of Maryland	
L.B. Puchall, M.D.	University of Maryland	

COOPERATING UNITS (if any)

University of Rochester, Rochester, NY
University of Maryland, College Park, MD

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Auditory and visual average evoked potentials (EPs) are being studied in a sample of identical twins given a placebo-controlled amphetamine trial. Genetic, hemispheric lateralization and reliability of drug effects will be assessed.

Research strategies in biological psychiatry were reviewed with emphasis on genetic approaches and methodological problems in group comparisons.

PHS-6040
(Rev. 10-76)

Project Description

The role of heredity in psychiatric illness is supported by the many studies demonstrating excessively high rates of illness in persons with a genetic relationship to known patients. The clinically variable expression of psychiatric disorders and the difficulty in identification of mildly affected relatives point to the need for biological markers for the genetic vulnerability. The heritability of AER measures combined with their applicability to both ill and well relatives makes them a promising approach. Further, differences have been demonstrated between patients with schizophrenia and various types of affective disorders.

Genetic mechanisms of the AER have been pursued this year in studies of pharmacological effects of amphetamine in normal volunteers. Thirteen pairs of Mz twins have so far been tested on placebo controlled double-blind trials. Eight twins received two placebo test days to assess test-retest reliability in addition. Data is currently under analysis.

A new collaborative study with Dr. R. Cromwell of the University of Rochester is being initiated. This program project has the assumption that deficits (and other variants) linked to schizophrenic vulnerability may be more useful than global clinical diagnostic variables in investigating genetic factors associated with that disorder. These vulnerability-linked variants tend to be identifiable (1) before, during, and after active episodes of schizophrenic illness, (2) in healthy first-degree relatives, and/or (3) in risk populations with personality traits suggestive of later schizophrenic breakdown. Another assumption is that better genetic understandings of schizophrenia may be obtained from the study of extended families both with (a) multiple and (b) single known instances of schizophrenia, together with (c) a pathology-free control group.

Large pedigree families will be examined with focus primarily upon five schizophrenia-related deficits. Of these, two are behavioral (reaction time crossover, span of apprehension with forced-choice/partial-report procedure), two are biochemical (blood platelet monoamine oxidase and plasma dopamine beta hydroxylase), and one is electro-physiological (visual evoked response augmenting/reducing). Each measure will first be analysed independently and blindly of clinical symptoms of the index family member. Wherever genetic segregation patterns are established for a variant, linkage studies will be conducted. In addition to the genetics analyses, intercorrelation and group comparison of the deficit variables in the experimental groups will be made. Finally, the subjects at extremes on the five experimental variables will be examined blindly on clinical symptom variables (current and historic) and for clinical diagnosis.

The genetic data analysis is based upon the strategy that susceptibility of disease can be studied in the distribution of "affected" and "unaffected" individuals for the disease-related traits within families where a single Mendelian dominant pattern of inheritance can potentially be fitted to the data. Since this pattern is expected to involve ad hoc assumptions about lack of penetrance and variable expressivity, the fit will invariably represent a combination of genetic and environmental factors. Linkage of the specific variants to marked loci with known Mendelian inheritance will then be examined. If the hypothesized genetic transmission of the deficits is not proximal to known marker loci, no linkage will be found. On the other hand, clear evidence of linkage with a marker locus will establish the validity of the hypothesized mode of transmission in that family.

Publications

Buchsbaum, M.S. and Gershon, E.S.: Genetic factors in EEG, sleep and evoked potentials. In Davidson, R.J. and Davidson, J.M. (Eds.): The Psychobiology of Consciousness, New York, Plenum Press, 1980, pp. 147-168.

Buchsbaum, M.S. and Rieder, R.O.: Biologic heterogeneity and psychiatric research. Platelet MAO activity as a case study. Arch. Gen. Psychiatry 36: 1163-1169, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00038-06 BP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Laterality, Psychopathology and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

none

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Visual AERs from the left and right temporoparietal and occipital cortex were recorded using visual stimuli presented to both visual fields simultaneously. Subjects included normal controls and patients with affective illness. Attentional enhancement of EP amplitude was most pronounced for the N120 component, left hemisphere lead, and this was accentuated by amphetamine. Normal controls have also been tested and data analysis is underway.

PHS-6040
(Rev. 10-76)

Project Description

The demonstration of hemispheric asymmetry in cerebral activity has proven to be a complex phenomenon dependent in part on such factors as the nature of the experimental stimulus or task, the sex, handedness and cerebral dominance of the subject, and the attentional and motivational conditions under which the stimulus is presented. The involvement of attentional factors is particularly intriguing in its implications for the pathophysiology of clinical syndromes in which such factors are disturbed and for the action of pharmacologic agents known to affect attention. Although pharmacological effects on hemispheric asymmetry are receiving more attention, relatively few studies of specific drug actions have been reported.

Nine patients (5 males, 4 females) were hospitalized at the National Institute of Mental Health for major depressive illness. Subjects were right handed and right eyed dominant and had been drug free for at least two weeks. Each patient was recorded approximately one hour after receiving either 20 mgm of d-amphetamine or saline placebo intravenously. The order of active drug and placebo infusions was randomly determined and experimental sessions were separated by an interval of at least 48 hours. Double-blind behavioral ratings by the subject and attending physician were completed prior to the drug administration and just before evoked response testing. EEG was recorded from right and left temporoparietal area referenced to ipsilateral ear, amplified, filtered and averaged on line. The baseline for measurement of N120 (116-152 msec) AER amplitude was determined from the mean value of all vertical coordinates within the evoked response. The mean of all absolute deviations from this baseline constituted the measure of amplitude of response.

Four intensities of light (2, 30, 80 and 240 foot lamberts) and four intensities of 500 Hz tones (50, 60, 70 and 80 dB) were presented 64 times each for a total of 512 presentations.

Each subject received the same sequence of light and tone stimuli for three runs (attend to lights, attend to tones, distraction with mental arithmetic). During the "light attention" condition, the subject was instructed to count the number of times consecutive light flashes were of the same intensity and told to disregard any intervening tones. Under the tone attention condition, the instructions were the same with the exception that tones were to be attended to and intervening lights ignored. During the mental arithmetic condition, the subject was asked to concentrate on counting backward by subtracting 7 serially from 2019; lights and tones were to be ignored although the subject was instructed to keep his eyes open. For each condition subjects were informed that the number of patterns counted would be recorded. Nearly all subjects were correct with ± 6 of the correct number.

The magnitude of the attentional enhancement on amphetamine and placebo was then compared, using two way ANOVA (intensities by attentional differences) calculated separately for each component and hemisphere. Greater amplitude increases with attention to the stimuli with amphetamine were statistically confirmed for component N120 for visual stimuli.

Attentional enhancement of EP amplitude appeared more pronounced over the left hemisphere lead. Further analysis of the components of this finding shows that the amplitude of visual EPs is enhanced by amphetamine over the left hemisphere under both attention to light and attention to tone conditions; although responses to attended light stimuli are similarly enhanced by amphetamine over the right hemisphere, the EP amplitude is not enhanced when tones are relevant. Thus, N120 enhancement is modality specific in the right but not left hemisphere.

Since numerous investigators have theorized that affective illness may be associated with a shift in hemispheric dominance, it should be emphasized that the population studied may have differed from a normal population in terms of differential hemispheric evoked response at baseline. As such, the reported response to active drug may be unique to this group. It is possible to speculate along these same lines that the emotional arousal commonly associated with amphetamine administration is related in part to the degree of right hemispheric activation reflected in certain components of evoked response. This hypothesis would be compatible with studies demonstrating greater right than left activation under conditions of emotional stimulation. Although the present study did not reveal an association, the smallness of the sample size and the inclusion of both sexes in the analysis would appear to limit the conclusions that can be drawn.

Publications

Reus, V.I., Buchsbaum, M.S. and Post, R.M.: D-amphetamine: Differential effects on right and left hemispheres. In Gruzelier, J.H. and Flor-Henry, P. (Eds.): Hemisphere Asymmetries of Functions in Psychopathology. Amsterdam, Elsevier, 1979, pp. 329-338.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00039-06 BP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Sensory Thresholds and Averaged Evoked Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	F. K. Goodwin, M.D.	Chief, Clin. Psychobiology Branch	CP NIMH
	R. M. Post, M.D.	Chief, Section on Psychobiology	BP NIMH
	D. L. Murphy, M.D.	Chief, Clin. Neuropharmacology Branch	CN NIMH
	M. Webster	Research Assistant	CP NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH Biological Psychiatry Branch

SECTION Clinical Psychophysiology

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.3	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Average evoked responses (AER) to varying intensities of electrical stimulation to the forearm and subjective ratings of these stimuli have been studied in 155 age and sex matched normal volunteers. The effect of morphine in normals and naltrexone in schizophrenics was also studied.

PHS-6040
(Rev. 10-76)

Project Description:

Pain and discomfort have long been known as the constant companions of psychiatric illness. Do patients with major affective illness and schizophrenia have a disturbed perceptual experience of pain as a result of their illness? What are the effects of major psychotropic drugs on pain? Are psychotropic drugs analgesics? In this era of neuropsychopharmacology, these questions can be more effectively approached than before. Many of the neuronal and behavioral actions of these drugs are now known and, in addition, we are beginning to understand the neuronal mechanisms for integration of pain. Thus, particular emphasis on the psychological aspects of pain in future research is desirable.

A group of 153 normal volunteers were ranked on insensitivity scores obtained on a psychophysical pain rating session. Somatosensory evoked potentials (EPs) were recorded to four stimulus levels ranging from barely perceptable to very unpleasant. The evoked potentials of individuals in the upper and lower quartile (39 and 38 individuals, respectively) of psychophysical pain ratings were then compared in order to maximize differences. The quartile limits were error rates of 15% and above classified as pain insensitive and 7.5% and below classified as sensitive. Examination of the mean EP waveforms for the groups reveals greater N120 and P200 components at high intensities in the sensitive individuals with little difference between groups for the lowest intensity.

Psychophysical pain ratings and somatosensory evoked potentials (EPs) were also studied in 17 off-medication patients with schizophrenia and 17 age- and sex-matched normal controls. Five of the 17 schizophrenic patients also participated in a clinical trial of naltrexone. In comparison with normal controls, schizophrenic patients were significantly more insensitive to painful stimulation (based on nonparametric analogues of d' from signal detection analysis) and had significantly smaller somatosensory EPs to painful stimuli for the N120 component. Schizophrenics treated with naltrexone showed significant increases in EP amplitude at higher stimulus intensities and hyperalgesic effects on pain ratings.

Twenty-six schizophrenics and 16 age- and sex-matched normal controls also participated in a study of selective attention using auditory and visual stimuli. Both visual and somatosensory EPs showed reduced N120 amplitude/intensity functions in schizophrenic patients as compared with age- and sex-matched normal controls. In the attention paradigm, the normal subjects showed significantly greater enhancement of N120 auditory amplitude with attentional instructions than did schizophrenics. This was more apparent at low stimulus intensities than at high stimulus intensities. Visual EPs showed the same trend but the difference between groups did not reach statistical significance. It should be noted that neither the P100 nor P200 components showed a significant difference between schizophrenics and normals' EP amplitude enhancements.

Our hypothesis, postulating a relationship between attentional deficits and peptide responsive symptoms in schizophrenia, also receives support in the analysis of the association between individual differences in EP attentional enhancement and pain sentivity as assessed by d'. Among normal individuals, the enhancement of auditory EP amplitude with attention to the auditory modality was significantly correlated with first session pain ratings ($r = 0.382$, low intensity and $r = 0.558$, high intensity, both $p<0.05$).

It is noteworthy that this correlation appeared with the auditory EPs where we observed a significant normal/schizophrenic difference in attentional effect and not in the visual EP where no such significant difference was found.

Earlier studies of effects of morphine and naloxone on EPs also showed N120 changes. Across all of these studies it has been the N120 component which has consistently shown opiate analgesia and attentional effects; taken together this is consistent with dysfunctional endorphinergic regulation of attention.

Publications:

Gillin, J.C., Sitaram, N., Mendelson, W.S., Buchsbaum, M.S., Moore, A.M., Jacobs, L.S. and Wyatt, R.J.: Cholinergic modulation of sleep, growth hormone and analgesia in normal man. In Jenkin, D. and Usdin, E. (Eds.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press, 1978, pp. 781-790.

Buchsbaum, M.S., Lavine, R.A., Davis, G.C., Goodwin, F.K., Murphy, D.L., and Post, R.M.: Effects of lithium on somatosensory evoked potentials and prediction of clinical response in patients with affective illness. In Gershon, S., Kline, N.S., and Schou, M. (Eds.): International Lithium Conference: Controversies and Unresolved Issues. Excerpta Medica, ICS Series, 1979, pp. 685-702.

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Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Research in endorphins and schizophrenia. Schizophrenia Bulletin, 5: 240-250, 1979.

Davis, G.C., Bunney, W.E., Jr., Buchsbaum, M.S., DeFraites, E. G., Duncan, W., Gillin, J.C., van Kammen, D.P., Kleinman, J., Murphy, D.L., Post, R.M., Reus, V. and Wyatt, R.J.: The use of narcotic antagonists to study the role of endorphins in normals and psychiatric patients. In Usdin, E. and Bunney, W.E., Jr. (Eds.): Endorphins and Mental Health Research. New York, MacMillan, 1979, pp. 393-406.

Haier, R.J., Rieder, R.O., Khouri, P.J. and Buchsbaum, M.S.: Extreme MMPI scores and the research diagnostic criteria: Screening males for psychopathology. Archives of General Psychiatry, 36: 528-534, 1979.

Lipper, S., Murphy, D.L., Slater, S. and Buchsbaum, M.S.: Comparative behavioral effects of clorgyline and pargyline in man: A preliminary evaluation. Psychopharmacology, 62: 223-128, 1979.

Rey, A. C., Savard, R. J., Silber, E., Buchsbaum, M. S., and Post, R. M: Cognitive changes and the averaged evoked response in depression. Cognitive Therapy and Research, 3: 263-267, 1979.

Davis, G. C., Buchsbaum, M. S., van Kammen, D. P., and Bunney, W. E. Jr: Analgesia to pain stimuli in schizophrenics reversed by naltrexone. Psychiatry Research, 1: 61-69, 1979.

Davis, G. C., Buchsbaum, M. S., and Bunney, W. E. Jr: Pain and psychiatric illness, In Ng, L. and Bonica, J. (Eds.): Pain, Discomfort and Humanitarian Care. Amsterdam, Elsevier, (In Press).

Davis, G. C., Buchsbaum, M. S., and Bunney, W. E., Jr. Endorphins: Endogenous control of the perception of pain. In Wain, H. and Devanis, D. (Eds.): Current Concepts in Pain, Springfield, Illinois, Charles Thomas, (In Press).

Buchsbaum, M. S., Davis, G. C., and van Kammen, D. P. Diagnostic classification and the endorphin hypothesis of schizophrenia: Individual differences and psychopharmacological strategies. In Baxter, C. F. (Ed.): VA Advisory Conference on Chronic Schizophrenia, New York, Raven Press, (In Press).

Davis, G. C., Buchsbaum, M. S., and Bunney, W. E. Jr. Alterations of evoked potentials link research in attention dysfunction to peptide response symptoms of schizophrenia. In Trabucchi, M. (Ed.): Advances in Biochemical Psychopharmacology. New York, Raven Press, (In Press).

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Davis, G. C., Buchsbaum, M. S., Naber, D., and van Kammen, D. P. Effects of opiates and opiate antagonist on somatosensory evoked potentials in patients with schizophrenia and normal adults. In Perris, C., and von Knorring, L. (Eds.): Recent Advances in Biological Psychiatry, Basel, Switzerland, S. Karger AG, (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00040-05 BP

PERIOD COVERED October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Amphetamine Pharmacokinetics and the Average Evoked Response in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G. L. Brown, M.D.	Medical Officer, Unit on Childhood Mental Illness	BP	NIMH
	J. L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
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COOPERATING UNITS (if any)

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LAB/BRANCH Biological Psychiatry Branch

SECTION Clinical Psychophysiology

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of amphetamine on the average evoked response (AER) is being studied in hyperactive children and normal adults.

PHS-6040
(Rev. 10-76)

Project Description:

Previous research has demonstrated changes in average evoked responses (AER) with administration of amphetamine in children and in patients with affective disorders. Individual differences are prominent both in behavioral response as well as the AER; some individuals are aroused and others show sedation or no effect and AERs both predict and parallel these differences. We examined the behavioral, cognitive and EP effects of a single dose of amphetamine in 14 normal prepubertal boys, 15 age matched hyperactive boys, two groups of adults tested at different dosages and in 16 adult Mz twin pairs (see Project Z01 MH 00037-08 BP), in a double blind study. When amphetamine was given, the normal boys showed a marked decrease in motor activity, improved vigilance performance and EP shifts away from augmenting. Very similar results, especially for EPs, were obtained in hyperactive children. The similarity of the response to amphetamine in normal and hyperactive children cast doubt on the concept of a clinically specific or "paradoxical" response to stimulants in this syndrome. For both vigilance and EP data, age affected EP results more than diagnostic group membership; age changes suggested delayed maturation in hyperactive children.

During the next year, data analysis and summary will be done on the multiple groups of individuals tested.

In a related project, an improved and more powerful measure of vigilance based on the continuous performance task was developed, using signal detection theory. Reanalysis of the data comparing hyperactive and normal children confirmed the above findings. These new measures proved reliable individual traits in test-retest trials. Given the reliability, sensitivity to amphetamine and age effects, this new measure formed the basis of a screening study for attentional dysfunction. Four hundred normal college students were tested and the upper and lower 5% recalled for psychiatric histories, childhood hyperactivity assessment, neurological, psychological and psychophysiological tests. A high followup rate was obtained (93% of the 43 high-risk subjects) and the studies completed by a team of blind testers. Data is currently under analysis and will be reported next year.

Publications:

Rapoport, J. L., Buchsbaum, M. S., Weingartner, H., Zahn, T. P., Ludlow, C., Bartko, J., Mikkelsen, E. J., Langer, D. H., and Bunney, W. E. Jr. Dextroamphetamine: Cognitive and behavioral effects in normal and hyperactive boys and normal adult males.

Archives of General Psychiatry, (in press).

Weingartner, H., Rapoport, J. L., Buchsbaum, M.S., Bunney, W. E. Jr., Ebert, M. H., Mikkelsen, E. J., and Caine, E. D. Cognitive processes in normal and hyperactive children and their response to amphetamine treatment. Journal of Abnormal Psychology, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00111-06 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Drug Abuse Studies/Stimulant Abuse/Opiate Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Irl Extein, M.D.	Guest Worker	CP,NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Studies of Drug Abuse

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.0	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project studies the biochemistry of mood alterations induced by drugs of abuse. Several drugs are under investigation: amphetamine, ethanol, and opiates. Research strategies include the measurement of opioid activity in plasma during acute drug administration and during withdrawal states. Further research strategies are currently under evaluation.

Objectives

A major effect of drugs of abuse in general is the production of alterations in mood. While drugs such as ethanol, amphetamine, and opiates differ pharmacologically, they share a common clinical effect: the production of an enhanced state of well being. This "high" is a major target of our investigation. Since opiate alkaloids are thought to produce their behavioral and biologic effects via binding to the same opiate receptor as do the naturally occurring opioid peptides (endorphins), it is hypothesized that endorphins may mediate other drug-induced states. To date, the phenomena of dependence, tolerance and withdrawal, well known aspects of opiate alkaloid pharmacology, have not been related to the endorphin system. Recent reports have suggested that the psychomotor depressant effects of ethanol can be blocked by naloxone administration, suggesting possible relationships between ethanol intoxication and endogenous opioids. The activation effect of amphetamine in laboratory animals has been blocked by naloxone. The interrelationships among amphetamine, dopamine, psychopathology and endorphins are currently under investigation.

Methods Employed

1. In collaboration with the Section on Psychogenetics, we are studying the effects of acute amphetamine administration on plasma levels of endogenous opioids using radioreceptor assay techniques. This study is currently in progress and may potentially provide knowledge of the relationship between opioids and the "high" produced by amphetamine.

2. We are currently studying the effects of acute ethanol administration on plasma level of endogenous opioids using radioreceptor assay techniques. We are further studying the relationship between ethanol and the opiate receptor in animal models. The Unit is currently evaluating the possibility of utilizing naloxone administration to reverse acute or pathological intoxication.

3. In collaboration with Dr. Irl Extein of Fair Oaks Hospital, we are studying plasma levels of opioid activity in addicts prior to and during heroin withdrawal. We are examining the effects of the non-opiate drug clonidine, which is useful in opiate detoxification, on plasma levels of opioid activity.

Major Findings

At the present time, these projects are in their initial phases. Our preliminary data suggests that acute ethanol administration produces an increase in plasma opioid activity.

Significance to Biomedical Research

Drug abuse is a significant problem. A basic understanding of the mechanisms of action of drugs of abuse could lead to pharmacological agents useful in prevention, detoxification, withdrawal and long-term treatment.

Proposed Course

These areas of investigation are all currently in progress. The major goal of this project is to gain biochemical understanding of the subjective "high" produced by drugs of abuse. At the present time we are studying the endorphin system and its relationship to the pharmacologic behavioral effects of these drugs. These projects will be ongoing during the coming year.

Publications

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00112-03 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Endorphin Research in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Richard Wagner, M.D.	Staff Psychiatrist, Laboratory of Clinical Psychopharmacology	DSMR,NIMH
Philip Gold, M.D.	Chief, Unit on Neuroendocrinology	LCS,NIMH
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Glenn C. Davis, M.D.	Guest Worker	BP,NIMH

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, DCBR, NIMH
Division of Special Mental Health Research, NIMH
Laboratory of Clinical Science, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	1.0	1.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is known that there are naturally occurring opiate-like peptides (endorphins) which appear to function as neurotransmitters and neuromodulators within the central nervous ystem. The major goal of this project is to study the possible neurobiologic role of endorphins in human behavior and psychiatric illness. The Unit has tested the behavioral effects of the narcotic antagonist, naloxone, and of beta-endorphin in affectively ill and schizophrenic patients. A sensitive radioreceptor assay to determine opioid activity in human CSF and plasma has been developed. This assay is currently being used to study the physiologic role of endorphins in man and possible disturbances in psychiatric patients.

Objectives

The major objective of this project is to seek understanding of the role of endorphins in normal neurophysiology, behavior, and mental illness.

Methods Employed

The following research strategies have been used to study the role of endorphins in human behavior and mental illness.

1. Narcotic antagonist treatment. Depressed and schizophrenic patients from the Clinical Center and St. Elizabeth's Hospital have been acutely administered the narcotic antagonist, naloxone, accompanied by careful clinical ratings to delineate possible behavioral effects of this drug. This Unit has served as the coordinating center for the World Health Organization study of naloxone in schizophrenia and mania project.

2. The direct administration of opiate alkaloids. In collaboration with the Clinical Psychobiology Branch and Nursing Unit 4-west, we have administered intravenous methadone to depressed patients to study the acute behavioral effects of opiates in affective illness and to study the neuroendocrine responses to opiates in these subjects. In addition, we have begun oral trials of methadone to test antidepressant efficacy.

3. Beta-endorphin administration. In collaboration with Nursing Units 4-west, 4-east and St. Elizabeth's Hospital, we have completed a pilot study of the behavioral and biologic effects of acute beta-endorphin administration in psychiatric patients.

4. Radioreceptor assay of opioid activity. A sensitive radioreceptor assay to determine levels of opioid activity in CSF and plasma has been developed. This assay is being used to study the possible relationships between endorphins and psychiatric illness. In addition, the technique is being used to study the effects of psychopharmacologic drugs and the endorphin system.

Major Findings

1. Previous studies performed in this Unit suggested that acute intravenous administration of naloxone produced improvement in the "thought disorder" subscale of the BPRS in schizophrenics, but no significant improvement in manic patients. The World Health Organization Naloxone in Mania and Schizophrenia Project, coordinated by the Unit, suggest that there may be a subgroup of schizophrenic patients who show such a positive therapeutic response to naloxone although this study does not demonstrate a therapeutic effect in the schizophrenic group as a whole. Manic patients showed consistently no improvement following naloxone.

2. Five milligrams of methadone was administered intravenously to seven depressed patients. There was no acute antidepressant effect. Several neuroendocrinologic effects were seen: prolactin was increased, cortisol decreased. This model may be useful in studying neuroendocrine-endorphin relationships. In the one patient who has completed an oral methadone trial, a rather marked antidepressant effect was observed during the second to third week of treatment.

Urinary-free cortisol excretion was reduced significantly during the first week of treatment with apparent "break-through" of this suppression as treatment went on, perhaps representing a biologic manifestation of opiate tolerance.

3. Six schizophrenic and four depressed patients received acute intravenous beta-endorphin administration. Three schizophrenics were observed to have marked behavioral effects--two worsening and one improvement. There were no clinically significant behavioral effects among the depressed patients. On the basis of plasma prolactin and cortisol responses and radioreceptor assay for opioid activity, strong evidence that infused beta-endorphin exerts opiate-like effects in vivo was gained.

4. We have found that plasma opioid activity in one cycling manic-depressive patient was significantly elevated during mania in comparison to depression. This assay has been used to determine opioid activity in CSF samples from schizophrenic, manic, and depressed patients as well as from patients with anorexia nervosa and with character disorders. The data analysis is currently taking place. We have observed in normal volunteers a diurnal rhythm in plasma opioid activity, and a similar rhythm in CSF opioid activity in monkeys. This pattern is similar to that of ACTH in so far as secretion appears to be episodic with elevated morning levels. We have also shown that hand immersion in water is associated with increases in plasma opioid activity supporting an association between pain and endorphins. Studies using plasma opioid activity to study pain and stress are ongoing.

Significance to Biomedical Research

Endorphins may play an important physiologic role in man. Results from narcotic antagonist treatment of psychiatric patients suggest that there may be a subgroup of schizophrenic patients who are responsive to naloxone administration. The radioreceptor assay technique appears to be an important tool for the study of endorphins in psychiatric illness and other behavioral states. Preliminary results suggest a relationship between endorphins and pain.

Proposed Course

Continuing studies include the assay of CSF and plasma opioid activity in human subjects. The Unit will continue to study the physiologic roles of endorphins using neuroendocrinologic and pharmacologic strategies. The Unit is currently evaluating the strategy of intrathecal administration of beta-endorphin in patients with intractable pain secondary to metastatic disease to evaluate potential analgesic effects.

Publications

Naber, D., Pickar, D., Dionne, R.A. et al.: Assay of endogenous opiate receptor ligands in human CSF and plasma. Alcoh. Act. Misuse 1: 85-92, 1980.

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Davis, G.C. and Bunney, W.E., Jr.: Studies of the role of endorphins in normals and psychiatric patients. Conference of the Combined Clinical Staffs, NIH, Ann. Intern. Med. 91: 246-248, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00113-03 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Psychophysiological Studies of Pain and Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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David Pickar, M.D.	Staff Psychiatrist, Unit on Studies of Drug Abuse	BP,NIMH

Other:

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LAB/BRANCH
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SECTION
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TOTAL MANYEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into Project Z01 MH 00039-06 BP.

PHS-6040
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00070-07 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Psychological and Biological Interactions in the Acute Psychoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study focuses on the longitudinal clinical, biochemical, and physiological evaluation and treatment of mania, depression, schizoaffective illness and related anxiety disorders and psychoses. In double-blind, placebo-controlled clinical trials we have found the temporal lobe-limbic anticonvulsant carbamazepine useful in the treatment of both phases of manic-depressive illness. Drugs such as piribedil, pimozide, and clonidine, which have effects at catecholamine receptors, also appear to have clinical and theoretically important effects in patients with affective illness. Neurotransmitter amine, endocrine, and peptide substances implicated in the regulation of mood and behavior are studied and measured in plasma and cerebrospinal fluid of patients and normal controls as an approach to brain function. Animal models, such as kindling and cocaine sensitization, are studied as they provide new approaches to the mechanisms underlying progressive development of behavioral pathology and suggest new treatment strategies such as carbamazepine for affective illness.

I. Project Description

A. Objectives

This project is engaged in the multidisciplinary, longitudinal study and treatment of patients with a spectrum of acute psychoses, particularly involving mood disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interaction.

B. Methods Employed

1. Subjects

a. Patients who meet formal Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness are admitted to the 3-West Clinical Research Unit, Section on Psychobiology. A rating scale for atypicality of depression has been developed with Dr. E.K. Silberman to more adequately differentiate and subtype the heterogeneous group of patients who meet RDC for primary depressive illness.

b. Normal volunteers are also admitted to provide control data, as well as to assess the relationship of selected biological and psychological variables. Forty-three normal volunteers have already been studied with lumbar punctures and a battery of other biochemical, physiological, and psychological tests.

2. Psychological and Biological Evaluation

a. During an initial drug-free interval, patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation, including EEG monitored sleep, averaged evoked potentials, the Halstead Category test, and other tests of cognitive function. Behavioral data are collected in a double-blind fashion twice daily, both by a trained nursing team and by patient self-ratings. Nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) and Hamilton Depression Inventory three times weekly.

b. Motor activity is measured continuously every 15 minutes with a miniaturized activity monitor developed by Dr. T. Colburn. Temperature and self-ratings of mood are measured every two hours to study circadian and infradian biological rhythms. Sleep is studied in collaboration with Dr. J.C. Gillin and average evoked response in collaboration with Dr. M. Buchsbaum.

c. A "life chart" technique has been developed to plot number, severity, and interval between affective episodes, so that the development, recurrence, and progression of the illness can be assessed.

d. Clinical and neuroendocrine parameters are evaluated following administration of apomorphine, clonidine, thyrotropin releasing hormone, arginine, LHRH, and hypertonic saline.

e. The degree of apomorphine-induced hypothermia, endocrine change, mood and activation change, as well as side effects, is measured to provide an index of dopamine receptor function. Response to the cholinergic agonist arecholine is assessed, as well as abnormalities of cortisol regulation, using the dexamethasone suppression test.

f. Amine metabolites and urinary free cortisol are systematically evaluated using daily 24-hour urinary collections across clinical state changes on and off medication.

g. Amine metabolites, electrolytes, and peptides are also measured during ill and well intervals in blood and CSF (in collaboration with Dr. F.K. Goodwin).

3. Treatment

a. Treatment and clinical evaluation are begun in individual and group psychotherapy, as well as ongoing clinical case conferences.

b. Both routine and experimental compounds are evaluated during double-blind clinical trials. Routinely used drugs including tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics are studied, not only for clinical efficacy, but to further understand their mechanisms of action.

c. The anticonvulsant carbamazepine is given acutely and chronically to patients with affective illness as a new treatment protocol.

d. Several experimental drugs with relatively specific receptor effects are also studied. A dopamine receptor agonist and antagonist, piribedil and pimozide, are used to dissect the role of dopamine in affective illness. Clonidine, an alpha-adrenergic agonist, and propranolol, a beta blocker, are also used.

e. Peptide mechanisms are explored using acute strategies with oxytocin and vasopressin, and chronic treatment with the vasopressin analog DDAVP.

f. The paradoxical acute antidepressant effects of one night's sleep deprivation in severely depressed patients are examined and possible underlying neurobiological and neuroendocrine changes are investigated.

4. Animal Models

A rodent and primate behavioral pharmacology laboratory is maintained to develop new research techniques in the longitudinal study of biobehavioral interactions. Particular focus is given to the study of chronic cocaine-induced behavioral pathology and the underlying changes produced by repetitive stimulation of the amygdala (electrical kindling).

C. Major Findings

1. Carbamazepine as a New Treatment for Manic-Depressive Illness

a. Clinical Efficacy. Studies of the clinical and biological effects of the temporal lobe-limbic anticonvulsant carbamazepine in patients with primary affective illness are continuing. Clinical trials in inpatients and outpatients are conducted in collaboration with Drs. J.C. Ballenger, T.W. Uhde, and W.E. Bunney. Studies of carbamazepine were initiated because of (a) reports of mood improvement in epileptic patients treated with the drug; (b) its particular usefulness in temporal lobe abnormalities, a putative substrate for affective regulation; (c) its ability to inhibit kindled limbic seizures; and (d) preliminary data in an open study in Japan reporting positive effect in manic-depressive illness. Carbamazepine has been evaluated in more than 30 inpatients, many of whom were previously lithium non-responsive. The drug has been acutely effective in more than half of the manic patients and slightly less than half of the depressed patients. A number of patients have shown unequivocal evidence of carbamazepine response, as indicated by repeated improvement during carbamazepine treatment, relapse upon placebo substitution, and renewal of clinical improvement when active drug is substituted.

In addition to its acute effects in both phases of affective illness, carbamazepine appears to have prophylactic effects against recurrences of manic and depressive episodes. Carbamazepine treatment has been continued on an outpatient basis in a small number of patients with dramatic reduction in episode frequency. One patient, chronically hospitalized in a state institution since 1956 with rapidly cycling manic-depressive illness, improved markedly during nine months of carbamazepine treatment as an inpatient and was able to be discharged to an outpatient status for 1½ years with only one manic recurrence.

b. Blood and CSF Carbamazepine Levels. Blood and CSF levels of carbamazepine are carefully monitored during the clinical trial. Blood levels, similar to those achieved during treatment for epilepsy (usually between 6 and 12 μg/ml) do not appear correlated with degree of clinical response. Levels of the parent compound of carbamazepine as well as an active epoxide metabolite have been measured in collaboration with Dr. Chattergy. The epoxide metabolite levels in plasma range between 6%-18% of those of the parent compound, while epoxide levels in cerebrospinal fluid are an average 23% of carbamazepine levels. Studies are in progress to establish whether the epoxide metabolite is related to degree of clinical response or side effects, as it has been reported to be an active anticonvulsant compound.

c. Studies of Carbamazepine's Neurotransmitter and Peptide Mechanism. A variety of studies are being conducted to elucidate possible mechanisms of action of the drug and to note alterations in neurotransmitter-related substances in patients during the clinical trial. Carbamazepine has a structure similar to the tricyclic antidepressant imipramine and appears to block the uptake of norepinephrine. CSF MHPG, a metabolite of norepinephrine, was significantly reduced during carbamazepine treatment, while urinary MHPG was unaffected. Initial studies of effects on dopaminergic systems suggest that probenecid-induced accumulations of homovanillic acid (HVA), a metabolite of dopamine, is decreased during carbamazepine treatment. However, the hormone prolactin, thought to be under inhibitory dopaminergic control, shows greater arginine-induced increases during carbamazepine treatment compared to the medication-free period. These findings, in collaboration with Drs. J.C. Ballenger and P. Gold, suggest that carbamazepine may have complicated effects on dopamine metabolism that do not resemble either simple dopamine agonists or antagonists.

Although alterations in gamma-aminobutyric acid (GABA) have been postulated to be important to carbamazepine's anticonvulsant effects, there was no significant effect of carbamazepine on CSF levels of GABA (measured in collaboration with Dr. T. Hare). Studies in animals suggest that carbamazepine may inhibit accumulations of cyclic AMP in a variety of preparations. Initial studies in collaboration with Drs. C. Smith and J. Tallman suggest that levels of cyclic AMP in CSF may be reduced in patients during carbamazepine treatment. These findings are of interest because several anticonvulsants, as well as lithium carbonate, have inhibitory effects on this system.

Carbamazepine has notable antidiuretic effects and has been used in the treatment of diabetes insipidus. It has been reported to both release vasopressin, as well as act as a direct vasopressin agonist. Consistent with these reports, Dr. T.W. Uhde has noted statistically significant and dose-related hyponatremia during carbamazepine administration; this effect is rapidly reversed on carbamazepine withdrawal. Drs. P. Gold and J.C. Ballenger have also noted decreased vasopressin response to a hypertonic saline load during carbamazepine treatment, a finding consistent with its possible direct agonist effects.

We have explored the possible interaction of carbamazepine with opiate peptide systems since carbamazepine is a useful treatment in several pain syndromes and has been reported to potentiate opiate-induced hyperactivity in mice. We found no significant effect of carbamazepine on CSF opioid binding activity measured in collaboration with Drs. D. Naber, D. Pickar, and associates. In 17 depressed and manic patients, medication-free values of opiate binding activity were 3.3 ± 0.4 picomoles/ml, while during carbamazepine treatment levels were 3.3 ± 0.5 picomoles/ml. However, in the 13 depressed patients, baseline pretreatment levels of opiate binding activity were positively correlated ($r = .79$) with the degree of clinical response. Thus, those with the highest levels of opioid binding activity in CSF were the ones who showed the greatest antidepressent response to carbamazepine. Other clinical and biological predictors of antidepressent response to carbamazepine are being investigated, although currently there is no adequate clinical or biological measure that differentiates those affectively ill patients who will respond to lithium carbonate from those who will respond to carbamazepine.

2. Noradrenergic Mechanisms in Affective Illness

On the basis of indirect pharmacological data in animals and man, alterations in norepinephrine have been postulated to occur during manic-depressive illness. In an effort to more directly assess this hypothesis we have measured norepinephrine, its metabolites, its biosynthetic enzyme, its effect on C-AMP, and have studied drugs which stimulate or block norepinephrine receptors.

a. Norepinephrine and MHPG in Urine, Plasma, and CSF of Affectively Ill Patients and Controls. Recent methodological advances have allowed us to directly measure the norepinephrine metabolite MHPG in plasma of patients during affective episodes (in collaboration with Dr. D.C. Jimerson). In several patients studied longitudinally, plasma MHPG was significantly higher during manic than during depressed phases, with virtually no overlap. These data are consistent with longitudinal studies of urinary MHPG which show changes in the same direction across clinical states. Cerebrospinal fluid norepinephrine, measured directly by sensitive radioenzymatic assay in collaboration with Dr. R. Lake, is also significantly elevated during the manic phase of the illness, both in comparison to the depressed phase and to normal volunteers. These findings are observed both during baseline, as well as probenecid procedures.

Although CSF norepinephrine is elevated during mania, activity of its biosynthetic enzyme dopamine-beta-hydroxylase (DBH), measured in collaboration with Dr. P. Lerner, is significantly lower in mania than in depression. This DBH-norepinephrine dissociation may lead to a better understanding of the specific synaptic alteration in noradrenergic mechanisms during mania. Although it is possible that differential transport out of CSF accounts for these findings, it is also possible that: (a) DBH could become depleted during increased firing; (b) there is a relative blockade of the reuptake of norepinephrine during mania; or even (c) a feedback inhibition of norepinephrine on its biosynthetic enzyme. In collaboration with Drs. D.C. Jimerson, J.C. Ballenger, and F.K. Goodwin we have studied the interrelationships of various measures of noradrenergic function in man. In normal volunteers, CSF and plasma MHPG are significantly correlated ($r = .70$, $p < .01$), while neither is correlated with urinary MHPG. CSF DBH and MHPG are also positively correlated in normal controls, and, in depressed patients, CSF levels of DBH and norepinephrine were positively correlated. These studies of many simultaneous measures of noradrenergic metabolism in different body fluids should help elucidate their normal relationships and their utility in assessing central nervous system, as opposed to peripheral, noradrenergic function.

b. The Alpha-Receptor Agonist Clonidine. Endocrine and behavioral responses to an acute intravenous challenge of clonidine are also being explored in order to assess possible alterations in noradrenergic receptor tone in psychiatric patients compared to normal volunteers. Clonidine is a potent agonist with apparent preferential effects on noradrenergic autoreceptors which result in inhibition of firing of norepinephrine cells. We are continuing trials of oral clonidine in manic and depressed patients to determine whether moderate doses of this agent might have antimanic and/or antidepressive effects. In addition, Dr. T.W. Uhde, in collaboration with Drs. E.K. Silberman, L. Siever, and D. Murphy, have infused clonidine (2 µg/kg i.v.) in 12 affectively ill patients and ten normal volunteers to investigate changes in behavior and MHPG, norepinephrine, beta-endorphin, and growth hormone. These data will provide information on the function of both pre- and postsynaptic noradrenergic systems in normal and affectively ill subjects. Preliminary data suggest that clonidine reduces anxiety in depressed patients. These data and other research have resulted in the design of a series of studies including a clinical trial of clonidine in phobic and panic disorders and, in collaboration with Dr. M. Buchsbaum, clonidine infusions in normal subjects to study noradrenergic mechanisms of pain modulation.

c. Noradrenergic Mechanisms in Normal Volunteers in Relation to Mood and Anxiety (with Drs. D.C. Jimerson, J.C. Ballenger, and W.E. Bunney). It was of interest in relationship to the hypothesized involvement of norepinephrine in affective regulation in manic-depressive patients to find that plasma MHPG was significantly and negatively correlated with Depression, Psychasthenia, and Hypochondriasis scales of the MMPI. To our knowledge this is the first report in carefully screened normal subjects that noradrenergic mechanisms may be associated with alterations in mood, with those having low MHPG having increased levels of depression and related symptoms. These findings would appear to be of clinical and theoretical interest in relationship to the possibility that normal variations in noradrenergic function are associated with normal variations in mood and behavior, as well as during psychopathological illness states.

We previously observed that depressed patients with higher anxiety levels had higher levels of norepinephrine in CSF. In the normal volunteers, high levels of CSF MHPG were also associated with increases in a measure of state anxiety. These findings are consistent with the recent work of Redmond and associates which suggests an important relationship between noradrenergic mechanisms in the locus coerulus and anxiety in the rhesus primate and man.

In a further replication in normals of the biological-behavioral associations noted in pathological patient groups, we observed a positive relationship between assaultiveness measured on the Buss-Durkee Scale with biological parameters such as cyclic AMP and MHPG in CSF. Dr. L. Brown and associates reported higher levels of CSF cyclic AMP and MHPG in aggressive patients; these relationships were again observed in this population which had no particular problems with aggression. Similarly, lower levels of CSF DBH have been associated with increased psychopathology on the MMPI in patients with personality disorders, as well as a predisposition to disulfiram psychosis in alcoholics. Normal volunteers with lower CSF DBH had higher Buss-Durkee irritability and higher trait anxiety scores in this population. Thus, not only did studies in these normal volunteers provide a control group for patients with manic-depressive illness, but began to address critical issues of the interrelationships of normal variations in behavior and biology.

3. Dopaminergic Mechanisms in Mania and Depression

Our previous studies have suggested alterations in dopaminergic mechanisms in affective illness based on the pharmacological evidence that piribedil, a dopamine agonist, was useful in some depressed patients, while pimozide, a dopamine receptor antagonist, was effective in treating manic patients. Measurements of the dopamine metabolite HVA were also consistent with this formulation, as manic patients treated with neuroleptics including pimozide, chlorpromazine, and thioridazine had greater HVA accumulations than did non-manic patients.

a. Apomorphine's Effects on Temperature and Endocrine Function in Patients and Controls. Apomorphine is also a dopamine receptor agonist which we have been using to study possible alterations in dopamine receptor function in man. Studies in collaboration with Drs. N.R. Cutler and W.E. Bunney have demonstrated diurnal variation in responsivity to apomorphine. Hypothermic responses were greater at 9:00 in the evening compared to 9:00 in the morning. However, there were no significant differences in mood, sedative, or endocrine effects at these two times. The hypothermic response to apomorphine may reflect alterations in dopamine receptor function at a hypothalamic level. These data are consistent with other observations of clinical alterations in hypothalamic dopamine function in animals and man. Repeated administration of apomorphine is associated with tolerance to hypothermic effects, but an exacerbation of the rebound hyperthermia. This differential effect may be related to altered responsivity in different dopamine receptors following repeated dopaminergic stimulation.

Male depressed patients had significantly lower baseline prolactin levels and greater prolactin reductions following apomorphine than male normal volunteers (studied in collaboration with Drs. D.C. Jimerson, A.C. Rey, N.R. Cutler, P. Gold, L. Brown, and W.E. Bunney). These findings are consistent with altered dopaminergic receptor function in depressed patients compared to normal controls. Apomorphine-induced growth hormone responses were not significantly different among the study populations, however.

b. State-Dependent Tardive Dyskinesia. Studies in collaboration with Drs. N.R. Cutler, A.C. Rey, and W.E. Bunney have for the first time documented that tardive dyskinesia may vary in association with mood state. That is, we have repeatedly observed in two rapidly cycling manic-depressive patients a marked exacerbation of dyskinesia during depressive phases of illness, with improvement and essentially disappearance of dyskinesias with the onset of mania. In addition to these dramatic and rapid changes with clinical state, gradual increases in dyskinesia toward the end of long manic episodes and gradual decreases in the marked dyskinesia toward the end of long depressive episodes suggest that slow alterations were occurring in the mechanisms underlying dyskinesias even within an episode. There were also diurnal variations in the appearance of tardive dyskinesia, with lower levels of dyskinesia found in the evening compared to morning during the manic phase of the illness.

The major theories of tardive dyskinesia have postulated dopamine receptor supersensitivity following neuroleptic withdrawal as the etiological mechanism. At the same time, there is indirect evidence of dopaminergic excesses in mania compared to depression. In combination, these current leading theories would predict that tardive dyskinesia would be more severe during mania than depression which was the opposite of what was repeatedly observed in our cycling patients. Thus, these observations would suggest a reconsideration of possible mechanisms underlying tardive dyskinesia or manic-depressive illness, and that one or both of these theories regarding dopaminergic mechanisms may require revision. In addition, the findings of state-dependent tardive dyskinesia (its marked exacerbation during depression and remission during mania) support the concept that biological changes capable of altering a neurological syndrome occur in association with alterations in mood and behavior in some patients with affective illness.

4. Endocrinological Alterations in Affective Illness and in Normal Volunteers

a. Cortisol Abnormalities Measured in Urine, Plasma, and CSF in Relation to Mood and Sleep Deprivation. In collaboration with Drs. P. Gold and J.C. Ballenger we have found significantly elevated levels of urinary free cortisol in bipolar and unipolar depressed patients compared to normal volunteers. Levels are significantly reduced in manic patients in comparison to both depressed patients and normal subjects. In addition, detailed longitudinal assessment of urinary free cortisol suggests that this parameter may change in close association with psychological state and in some instances cortisol elevations appear to precede the behavioral switch into depression. Dr. D.R. Rubinow has further documented a highly significant relationship between higher levels of plasma cortisol and depressive phases of a rapidly cycling manic-depressive patient. Again, these alterations in cortisol appear closely associated with clinical state and the switch process.

Alterations in the pituitary adrenal axis have also been studied using three other methodological approaches. In collaboration with Drs. T.W. Uhde, J.C. Ballenger, N.R. Cutler, W.E. Bunney, and E. Weitzman, we have observed marked cortisol hypersecretion in 20-minute plasma drug samples studied longitudinally in patients before, during, and after sleep deprivation-induced mood improvement and in one patient during a spontaneous switch into hypomania. These studies confirm previous reports of endocrinological abnormalities in depression and show a generalized hypercortisolism with lack of diurnal variation, i.e. no evening and night-time suppression of cortisol, as observed in normal volunteer controls studied concomitantly. It is of interest that in spite of this marked abnormality in cortisol secretion (similar to that observed in Cushing's disease), these patients did not show abnormal escape from dexamethasone suppression. We are currently enlarging our patient series in order to more precisely assess the relationship between hypercortisolism and escape from dexamethasone suppression (in collaboration with Drs. D.R. Rubinow and J.C. Gillin).

In the 20-minute catheter studies we also observed dysregulation of growth hormone secretion with absence of sleep-related growth hormone peaks in the depressed patients compared to normal volunteers. Plasma-free MHPGs were also significantly elevated during the course of the spontaneous switch into hypomania compared to depressed phases in this patient. These detailed longitudinal observations thus provide evidence for alterations in cortisol secretion and rhythm, as well as growth hormone rhythm and noradrenergic metabolism in affectively ill patients compared to controls. Cortisol measured in CSF in collaboration with Dr. P. Lerner was not significantly elevated in depressed patients compared to volunteers studied at 9:00 a.m. Further studies are in progress to elucidate possible differences in diurnal variations in CSF cortisol in depressed patients compared to normal volunteers. Taken together, our current data suggest there are major alterations in the regulation of the pituitary adrenal axis in patients with affective illness compared to controls and are consistent with one of the most replicable findings in the literature over the past two decades demonstrating such abnormalities in a variety of laboratory settings using many different techniques. Further studies will be designed to more specifically elucidate the mechanisms underlying this disturbed endocrine regulation in affective illness and its possible neurotransmitter concomitants.

b. Cortisol and Normal Variations in Mood and Behavior. In light of these findings in affective illness it was of particular interest to examine whether normal variations in mood would be associated with endocrine changes in the normal volunteers studied in collaboration with Dr. J.C. Ballenger. Consistent with the findings of low cortisol in mania, we found a significant inverse relationship between plasma cortisol of volunteers and the Mania scale of the MMPI. In addition, higher levels of urinary free cortisol were associated with increases in the MMPI Depression scale. Again, these findings in carefully selected normal volunteers suggest the possibility of similar psychobiological relationships occurring in the regulation of normal alterations in affect, as well as during more marked psychopathological states.

c. Opiate Mechanisms in Affectively Ill Patients and Normal Volunteers. In collaboration with Drs. D. Pickar, N.R. Cutler, D. Naber, C. Pert, and W.E. Bunney we have measured opiate activity in plasma of rapidly cycling manic-depressive patients, as well as in a large series of normal and affectively disturbed patients (with Dr. F.K. Goodwin) where opiate binding was assessed in CSF. A variety of data suggests that CSF opiate levels may be independent of plasma levels and reflect brain alterations in opiate activity more directly than those in the periphery. We found significantly elevated plasma opiate activity during mania compared to depression in a rapidly cycling manic-depressive patient. However, CSF opiate activity measured by an opiate binding assay was not significantly altered in depressed and manic patients compared to controls.

d. Vasopressin Alterations in Volunteers and Affectively Ill Patients. Vasopressin is a peptide with prominent effects on learning and memory, as well as possible effects on mood and cognition, in addition to its role in fluid regulation. Several approaches to studying vasopressin function have been initiated in collaboration with Drs. P. Gold, J.C. Ballenger, and H. Weingartner. Administration of the vasopressin analogue 1-desamino-8-d-arginine vasopressin (DDAVP) is associated with improvement in memory and recall in both affectively ill patients and in normal volunteers. CSF vasopressin has also been measured in collaboration with Drs. P. Gold and G. Robertson. Levels of CSF vasopressin were significantly lower in depressed patients compared to manic patients; these values bracketed those observed in normal volunteers. Vasopressin response to hypertonic saline appears to differ in the manic compared to the depressive state in rapidly cycling patients. Within the normal volunteer population CSF vasopressin levels were positively correlated with the measure of digit span recall on the WAIS test.

Although some positive effects on mood were noted following DDAVP administration in the initial patients studied, further clinical trials more clearly suggest that the improvement in memory and recall may occur independently of any improvement in mood.

e. Other Peptides in CSF: The CSF Pathway as an Active Neuroregulatory Link in Relation to Behavior. Almost all of the newly-discovered putative central nervous system peptides are present and measurable in CSF. In animal studies, alterations in the concentrations of peptides in CSF appear to exert effects on behavior and memory. We have completed an extensive review of this data and suggest that the CSF pathway should be explored in relationship to its active neuroregulatory function in addition to its transport and excretion. In addition to vasopressin, opiate compounds, and cortisol (mentioned above), we have been studying CSF somatostatin in collaboration with Drs. P. Gold, D.R. Rubinow, and S. Reichlin, calcitonin in collaboration with Drs. K. Becker and J.S. Carman, and angiotensin, as well as several enzymes in the angiotensin synthetic system, in collaboration with Drs. P. Gold and D. Ganten. It is hoped that these studies will not only provide information about possible relationships of these substances to affective disturbances, but also may help generate a basic physiology of the interrelationships among peptides in the central nervous system and of the regulation of normal physiology and behavior.

5. Clinical Phenomenology

a. Life Charting of Affective Episodes in Manic-Depressive Patients. We are near completion of a project to extensively present for pictorial and quantitative review data on the life course of manic and depressive episodes in affectively ill subjects. Using conservative cut-offs for the definition of episodes based on Research Diagnostic Criteria and prominent functional incapacity, we have charted the life course of manic and depressive episodes in most of the patients hospitalized in the Section on

Psychobiology over the past seven years. Accurate life charting of affective episodes utilizing maximal resources (including information from patients, nursing staff, physicians, social workers, family members, and previous hospital records) is a difficult and time consuming process, but appears to be extremely valuable from both a clinical and research perspective.

The course of illness delineated by this more detailed life chart process is markedly different from the perspective gained from considering only coarse measures of illness, such as number of prior hospitalizations. For example, there is no relationship between the number of depressive episodes that patients experience and the number of times they are hospitalized. In our patient cohort, bipolar patients compared to the unipolars have had a more severe course of illness prior to their NIH hospitalization as characterized by longer duration of illness, greater numbers of depressive episodes, more episodes per years ill, and more episodes in the year prior to NIH admission, but not a greater number of hospitalizations. It is also of particular interest that women tend to be faster cyclers and have a greater number of episodes in the year prior to NIH hospitalization compared to men. These data are consistent with a large literature suggesting that women may be disproportionately represented in populations of patients with extremely rapid cycling courses of illness. The endocrinological and neurotransmitter concomitants of this process are currently being explored in collaboration with Drs. P. Gold, D.C. Jimerson, and D.R. Rubinow. The life chart method also appears critical for accurate evaluation of the efficacy of the long-term treatment of patients with psychotropic drugs.

Dr. N.R. Cutler has also studied the inpatient hospital charts of six affectively ill subjects hospitalized for more than 25 years at St. Elizabeth's Hospital. These data give a detailed analysis of the consistencies and variations in the course of rapidly cycling and chronically hospitalized manic-depressive patients. The findings reveal marked heterogeneity in patterns of affective illness both within an individual's course and among different patients studied. These data not only re-emphasize Kraepelin's earlier findings, but are also interesting in relation to newer theories of underlying biological rhythm abnormalities in affectively ill patients. These biological theories must be comprehensive enough to deal with the marked variability in patterns of illness and not merely postulate a given underlying biological dysrhythmia which is constant throughout the illness. This study, as well as those in the literature, indicate that patients with recurrent episodes of either manic or depressive illness have a tendency for faster recurrences of episodes as their illness progresses. This apparent sensitization to further recurrences could be related to similar mechanisms occurring in drug sensitization and electrical kindling models, as discussed in more detail above.

b. Atypicality of Depression (in Collaboration with Drs. E.K. Silberman and F.W. Putnam). A rating scale for the typicality of depressed patients who would otherwise meet Research Diagnostic Criteria for primary affective illness has been constructed. We have been increasingly impressed with the heterogeneity of depressive presentations even within the group of patients defined by the above research criteria. The atypicality scale measures the degree to which patients diverge from a classical syndrome of affective illness with endogenous features and discrete episodes. It assesses degree of autonomy of symptoms, vegetative signs, personality disorder, neurotic overlay, bizarre thinking and perceptual disturbances, past course of illness, and premorbid adjustment which reflect alterations in interpersonal relationships, narcissistic entitlement, manipulativeness, prominent anxiety, or somatization. Older depressed patients were less atypical than younger patients, and bipolar patients were less atypical than unipolars. In addition, the atypicality dimension related to a variety of life course characteristics of illness with the more atypical patients having an increased total number of hospitalizations, as well as less rapid cycling in the year prior to NIH admission. The atypicality dimension appeared to have helped explain some of the biological variance noted within affectively ill subgroups. For example, atypical depressed patients have significantly greater variance in measures of noradrenergic metabolism including urinary MHPG and CSF norepinephrine than did the more typical patients. They also have a differential response to treatment, with typical patients having a higher incidence of positive response to sleep deprivation.

c. Psychosensory Phenomena in Association with Affective Illness. In collaboration with Dr. E.K. Silberman, we have developed a psychosensory rating scale designed to measure the degree and frequency of recurrence of a variety of phenomena usually associated with temporal lobe epilepsy. We have noted alterations in perceptual processing, orientation, and memory usually associated with psychomotor epilepsy occurring in patients with affective illness. Initial interviews conducted by Dr. E.K. Silberman suggest that patients with primary affective illness may indeed have a high incidence of these symptoms in a range similar to that seen in patients with epilepsy.

d. Other Psychological Tests, Hemispheral Laterality, and Averaged Evoked Response. A variety of other measures to assess learning, memory, and associated affective response are described in more detail by Dr. E.K. Silberman. One theme focuses on alterations in hemispheral laterality and the role that hemispheral specialization may play in disorders of affect and cognition. A vast literature has related the right, compared to the left, hemisphere in differential information and affective processing. Our studies are being conducted in parallel with lateralized average potentials (in collaboration with Dr. M. Buchsbaum). This data base in both patients and volunteers should provide important information about the relative lateralization of psychological as well as physiological information. We have noted differences in the laterality processing of information in depressed patients receiving amphetamine compared to controls (with Drs. V.I. Reus and M. Buchsbaum).

e. Abstracting Ability as Measured by the Halstead Category Test. In collaboration with Dr. R. Savard, depressed patients and controls have completed the Halstead Category test at various intervals. Striking alterations in cognitive function have been delineated in affectively ill patients compared to normal control and age- and socioeconomic status-matched spouse controls. Depressed patients have significantly greater impairment on the Halstead Category test during both their ill and improved states. In addition, the bipolar depressed patients, particularly those in the older age groups, have significantly greater Halstead measured deficits. Preliminary data also suggests that depressed patients with higher excretion of urinary free cortisol are the ones with the most prominent impairment of abstracting ability on the Halstead test.

6. Laboratory Models for the Progressive Development of Psychopathology

a. Kindling (in Collaboration with Dr. F.W. Putnam and N.R. Contel). We are continuing studies of the phenomenology and behavioral effects of repeated electrical stimulation of the brain. During kindling once daily stimulation of the amygdala results in increasing spread, duration, and complexity of after-discharges culminating in the appearance of major motor seizures to a previously subthreshold stimulation. The changes in electrical responsivity induced in this fashion are relatively permanent. Animals subjected to amygdala kindling also show long lasting alterations in pharmacological responsivity. They are significantly more likely to have lidocaine-induced convulsions, yet show decreased exploratory activity and are hyporesponsive to the psychomotor stimulant effects of cocaine. These data suggest complex interrelationships between the effects of repeated psychomotor stimulants, local anesthetics, and electrical stimulation of the brain.

b. Wet Shake Behavior During Kindling (in Collaboration with Dr. A. Pert and K. Squillace). We have for the first time documented and described in detail the occurrence of wet shake behavior following amygdala kindling. Wet shakes only occur following amygdala stimulation which result in after-discharges or seizures. They occur an average of 83 ± 2 seconds following amygdala stimulation and appear to be linked to alpha-adrenergic receptor mechanisms as they are suppressed by the alpha-agonist clonidine. They do not appear to be mediated by typical opiate systems as neither morphine nor naloxone pretreatment markedly affect wet shakes. Diazepam, which suppresses both kindled seizures and after-discharges, also eliminates wet shakes. It is likely that neurotransmitter or peptide effects associated with amygdala kindled after-discharges and seizures are mediators of the wet shake behavior.

c. Pharmacological Kindling. The concept of kindling has been expanded to include the development of seizures following repeated administration of drug as well as electrical stimuli. Over the past several years the convulsive and behavioral effects of repeated lidocaine administration have been explored. In collaboration with Drs. L. Sokoloff, C. Kennedy, and associates we have demonstrated that lidocaine seizures are selectively activating limbic system structures, particularly the hippocampus, amygdala, and perirhinal cortex as well as cingulate gyrus, as documented by increases in metabolic activity measured by the ^{14}C-2-deoxyglucose method.

1) Lidocaine Seizures and Aggression. In animals experiencing repeated lidocaine-induced limbic seizures a marked increase in aggressive behavior has been observed. These data suggest the usefulness of this model in dissecting the electrophysiological and neuroanatomical substrates involved in this type of aggressive behavior. The lidocaine limbic seizure model may thus be useful in exploring the relationship of repeated limbic and temporal lobe epilepsy to alterations in behavior in a well described animal model under controlled laboratory conditions.

2) Alcohol Withdrawal. The pharmacological kindling model is also of possible clinical relevance in relationship to alcohol withdrawal phenomena. In collaboration with Dr. J.C. Ballenger we noted that alcoholic patients early in their course of illness did not develop severe alcohol withdrawal symptomatology. In contrast, those later in the course of illness (presumably having experienced an increased number of alcohol withdrawal episodes) had increasingly severe withdrawal phenomena, including delirium tremens and, in some cases, withdrawal seizures. We suggested that repeated episodes of alcohol withdrawal may be acting as the kindling stimulus in patients with alcohol problems. This reconceptualization of alcohol symptomatology, as related to repeated episodes of withdrawal and encompassing a process that can be progressive, has obvious treatment implications. If valid, it thus becomes important to suppress withdrawal symptoms and possibly prevent their emergence. We have suggested, and preliminary data from studies in the European literature have supported the concept, that carbamazepine, a useful temporal lobe-limbic anticonvulsant, may be effective in treating alcohol withdrawal symptomatology. It may also prevent progressive development of a variety of psychopathological and convulsive manifestations in alcoholics undergoing repeated episodes of withdrawal.

d. Behavioral Sensitization to Psychomotor Stimulants. Behavioral responses to repeated administration of subconvulsive doses of the local anesthetic lidocaine and the local anesthetic-psychomotor stimulant cocaine continue to be explored in detail in the rat and rhesus monkey. The findings of a progressive development of behavioral pathology in response to the same dose are of interest in relation to both the development of paranoid psychosis in stimulant abusers and the basic alterations in neuronal pathways mediating this altered responsivity. We have recently reviewed the extensive literature regarding phenomenology and possible mechanisms underlying behavioral sensitization to the psychomotor stimulants and their relationship to the development of psychotic behavior.

1) Environmental Context Dependency. We have shown that repeated administration of cocaine leads to a behavioral sensitization effect which is in part context dependent. That is, animals repeatedly administered cocaine in the context of the test cage environment show greater hyperactivity and stereotypy than animals receiving the same doses of cocaine in a different cage. All animals received identical handling, cage experience, and numbers of cocaine and saline injections; the two groups differed only in order of injections (cocaine-saline compared to saline-cocaine) and where they experienced the cocaine hyperactivity. These

data further suggest important interactions of ongoing behavior and environmental context, as well as contingencies of the drug in relation to the environment, as critical determinants of the ultimate behavioral response in addition to the pharmacological effects of the drug considered in isolation.

2) Peptidergic Modulation of Behavioral Sensitization. We are conducting an ongoing series of studies to elucidate not only the neurotransmitter mechanisms underlying the behavioral sensitization effect but also the peptide modulators of such increased responsiveness over time. Since vasopressin has marked effects on learning and behavior in other animal paradigms we decided to explore the effects of vasopressin on the behavioral sensitization paradigm, as another model of learning and memory. Cocaine was repeatedly administered to Brattleboro animals, both homozygotes with a genetic deficiency in vasopressin leading to complete diabetes insipidus, as well as their litter mate heterozygote controls with normal levels of vasopressin and no diabetes insipidus. We found that behavioral sensitization to cocaine proceeded less rapidly in the homozygote (diabetes insipidus) animals compared to the heterozygote controls. In addition, the differential effects persisted in a long-lasting fashion following chronic cocaine administration. That is, the heterozygotes showed increasing response to cocaine administration at intervals of 3, 16, 21, and 37 weeks following chronic administration compared to the vasopressin-deficient homozygotes. These data suggest that vasopressin may be an important peptide modulator of the increasing behavioral response to repeated psychomotor stimulant administration. Further studies are planned to elucidate how vasopressin and other peptides are involved in the sensitization phenomena and whether different peptides, such as oxytocin with putative "amnesic" effects in other animal models, will impair behavioral sensitization.

3) Sex Differences in Behavioral Sensitization. Male compared to female rats show greater behavioral sensitization to the psychomotor stimulant cocaine. These differences may relate to a variety of neurotransmitter and hormonal differences between the two sexes and may be important in relation to the underlying interactions of sex hormones and dopamine receptor mechanisms. Female animals fail to show behavioral sensitization in part because of higher levels of hyperactivity and stereotypy at the outset of the experiment compared to male rats of similar age and weight. Findings of altered behavioral sensitization in females are consistent with the findings of H.L. Klawans and associates that females do not show the same increases in dopamine receptor hyperactivity following neuroleptic withdrawal.

4) Effects of Lithium on Cocaine Sensitization. In continued collaboration with Drs. A. Pert, C. Pert, and J. Rosenblatt the effect of lithium co-treatment on the development of behavioral sensitization has been studied. Lithium at clinically useful blood levels appears to impair the development of behavioral sensitization to cocaine and may be associated with altered adrenergic and dopaminergic receptor function.

e. Psychiatric Implications. As reviewed above, the kindling and behavioral sensitization paradigms may be useful for conceptualizing and studying selected mechanisms involved in the progressive development of psychopathology. In addition, we would question whether the repeated observations in manic-depressive patients of an increasing proclivity to recurrence of affective episodes is also a similar type of behavioral sensitization phenomenon. It is of particular interest that two of the drugs useful in preventing either behavioral sensitization (lithium carbonate) or electrophysiological kindling (carbamazepine) are also useful in the acute and prophylactic treatment of recurrent manic-depressive illness. It is not suggested that the kindling and behavioral sensitization paradigms are ideal models for progressive manic-depressive illness, but they may help in the consideration of new mechanisms underlying progressive aspects of the illness, as well as possible neuroanatomic and neurotransmitter pathways involved and new treatment approaches.

D. Proposed Course of Project

Studies of the clinical efficacy of carbamazepine in manic-depressive patients and other psychopathological states will continue. In addition, efforts will be made to clinically and biologically identify carbamazepine responders and to differentiate those characteristics which may predict carbamazepine, as opposed to lithium, response. Studies of carbamazepine's mechanism of action will continue both in our clinical populations using appropriate neurotransmitter and pharmacological challenge studies, as well as in the laboratory where techniques are being developed to administer carbamazepine acutely and chronically to animals.

The clinical studies of patients treated with relatively specific receptor agonists, such as clonidine and piribedil, and antagonists, such as pimozide, will be continued. These studies, in conjunction with concomitant measurements of the neurotransmitter effects of these compounds, should enhance our understanding of alterations in neurotransmitter pathways associated with affective illness and its amelioration with appropriate psychopharmacotherapies.

In collaboration with Dr. F.W. Putnam a study of procaine hydrochloride will commence in patient populations with affective and cognitive disorders in comparison to normal controls. Procaine appears to relatively selectively activate limbic structures and has been associated with positive effects on mood in some patient groups. The effects of procaine on mood, cognition, and EEG should help provide evidence of altered limbic activity among different psychiatric patient populations and (as reported for other symptoms) may provide a basis for identifying which patients will respond to the temporal lobe-limbic anticonvulsant carbamazepine.

The recent discovery of opiate and other peptides as putative central nervous system neurotransmitters appears to be one of the more dramatic advances in neurobiology in recent history. We hope to systematically explore the possible role of neuropeptide alterations in relation to affective disturbance and psychosis in a variety of ways, such as using peptide assays in man, as well as acute and chronic treatment strategies. For example, clinical trials of peptide substances will continue such as those involving DDAVP in collaboration with Dr. P. Gold. In addition, other acute challenges with peptides will be explored in relation to their cognitive and behavioral effects. In particular, the effects of oxytocin, as well as vasopressin, on learning, memory, behavior, and affect will be explored in collaboration with Drs. D.R. Rubinow and H. Weingartner. Arginine vasotocin will also be administered under similar conditions. Our collaborative efforts will continue in an effort to measure a variety of peptide substances in plasma and in particular in CSF in order to study normal interrelationships of these peptides with each other, with the more classical neurotransmitter amine substances, and ultimately to establish how they may relate to the regulation of normal and pathological behavior.

Under the direction of Dr. T.W. Uhde, studies of the baseline psychobiology and treatment of patients with phobic anxiety will be initiated. Following baseline drug-free clinical and biological evaluation, patients will participate in double-blind, placebo-controlled clinical trials of clonidine and carbamazepine in an effort to develop new treatments and to better understand underlying neurotransmitter mechanisms.

It is hoped that the developing battery of clinical and biological tests in patients with manic-depressive illness, anxiety, and related disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 00049-06 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Psychosocial Factors in the Prognosis of Melanoma
Formerly: Psychosocial Aspects of the Progression of Malignant Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Daniël P. van Kammen, M.D., Ph.D.	Unit Chief, Section on Neuropsychopharmacology	BP	NIMH
OTHERS:	G.N. Rogentine	Senior Investigator	I	NCI
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COOPERATING UNITS (if any)

Immunology Branch, NCI

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Neuropsychopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.6	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the effects of various psychosocial factors on host resistance to malignant melanoma. A detailed characterization of these factors in patients with melanoma, Clinical Stage II, is being obtained. In addition certain endocrine and immune parameters are also being studied. We are testing several hypotheses which may distinguish those patients suffering recurrence of their disease from others who either remain disease-free or have a delayed recurrence. At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO type, (3) social assets and (4) melanoma adjustment self-rating score.

In a second group of 30 melanoma patients, findings (1) and (4) were replicated as predictors of relapse within one year.

PHS-6040
(Rev. 10-76)

Project Description

Objectives

This study is aimed at developing a detailed characterization of the psychosocial factors which distinguish those patients who suffer recurrence of malignancy from those who remain disease-free or who have a delayed recurrence. Data is being gathered to test several current hypotheses concerning psychosocial antecedents of the onset of physical illness and predisposing psychobiological states.

Fifty to 100 patients with "surgically-cured" Stage II Melanoma will be studied. We plan a multi-level assessment of each patient. In addition to the biological measures gathered on the patient, we will characterize the patient's genetic history, past psychosocial history, level of personality development, current life stress and present psychological state. We will accomplish this by (1) psychiatric interview, (2) objective assessment of a five-minute speech sample, and (3) standardized questionnaires including MMPI, Holmes and Rahe Schedule of Recent Experience, SCL-90, Rotter Internal-External Locus of Control Scale, Beck Depression Inventory and the Gottschalk In-Out Scale.

The patients are seen shortly after their "curative" surgery and will be followed up at 2-month intervals, at which time we will obtain the Holmes-Rahe Scale, the SCL-90, Helplessness-Hopelessness Index and Beck Depression Inventory.

At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO types, (3) social assets and (4) melanoma adjustment self-rating score. We are presently examining this finding in a third group of patients. The ABO finding did not hold up but the melanoma adjustment score did.

Data analysis is underway.

Publications:

Fox, B.H., Boyd, S., van Kammen, D.P., Rogentine, G.N., Jr. Further analysis of psychological variables in predicting relapse after stage II melanoma surgery. Proceedings, Third International Symposium on Psychobiologic, Psychophysiologic, Psychosomatic, Sociosomatic Aspects of Neoplastic Disease, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00132-06 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Biological and psychopharmacological evaluation of schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
Clinical Branch, NEI; Immunology Branch, NCI; Laboratory of Clinical Psychopharmacology, NIMH; Laboratory of Clinical Science, NIMH; Laboratory of Psychology and Psychopathology, NIMH; Laboratory of Clinical Psychobiology, NIMH

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SECTION
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TOTAL MANYEARS: 14.0	PROFESSIONAL: 11.0	OTHER: 3.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the United States alone, it is estimated that 2 million people suffer from schizophrenia. Because of its very nature, this disorder is difficult to study. Pharmacological treatment is a particularly effective intervention in schizophrenia, but not a cure for all patients. Experimental and clinical findings have indicated that schizophrenia is a heterogeneous psychiatric disorder with biochemical, psychophysiological, psychological and genetic components. Patients are not constantly psychotic and their clinical state may change, regardless of medication conditions. However, we are now able to study a schizophrenic patient population intensively and longitudinally, with and without pharmacological approaches during different clinical states. We are beginning to ask fundamental questions about the nature of schizophrenia, indications for pharmacological treatment, vulnerability to psychotic decompensation and the role that genetics may play in the transmission of the disorder.

Our work integrates the multidisciplinary approaches in an effort to understand this complex disorder which may lead to a more effective treatment.

PHS-6040
(Rev. 10-76)

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Project Description

The Section on Neuropsychopharmacology (1) plans and conducts multidisciplinary research on the etiology and treatment of schizophrenia and (2) conducts research to define the biological dysfunction in schizophrenia.

Objectives

1. Multidisciplinary research on the etiology, treatment and prevention of schizophrenia.
2. Evaluation of the dopamine, other amine and peptide hypotheses of schizophrenia
3. Evaluation of potential markers of vulnerability and predictors of antipsychotic drug response
4. Psychopharmacological and biochemical evaluation of trait- and state-dependent variables of schizophrenia
5. Evaluation of novel treatments such as hemodialysis, propranolol and naltrexone.

Methodology

One to 14 days after admission in a double-blind fashion, patients are taken off their neuroleptic medication and put on placebo. After this 2-week "washout period," patients go through a 3 to 4-week test period in which diagnostic evaluation, medical and neurological workup, cerebrospinal fluid (CSF) collections, drug infusions (amphetamine, β-endorphin, placebo), psychological and psychophysiological testing and neuroendocrine evaluations take place. These are followed by two or more drug trials which include pimozide, lithium, diazepam, GHB, naltrexone, muscimol or propranolol. During any of these drug trials, testing similar to the admission test week can take place. Each drug period is separated by a 2-week placebo "buffer" period. The last of these periods is either followed by another test period or immediately followed by a 4-week clinial period where, if indicated, the patient is put on generally available antipsychotic medication and prepared for discharge. This extensive data-collection system allows us to evaluate the individual variables and drug responses against one another to observe the patient in different clinical states. It enables us to develop psychosocial, endocrine, biochemical and pharmacological profiles on patient groups defined by each variable.

A major problem of schizophrenia research has always been certainty of diagnosis. In addition to using the International Pilot Study of Schizophrenia criteria as developed by the World Health Organization and the former Psychiatric Assessment Section of the Adult Psychiatry Branch, we are applying the Research Diagnostic Criteria (RDC) as described by Dr. Spitzer and his collaborators to define diagnostically our patient population. This coming year, RDC will be replaced by somewhat more descriptive DSM III criteria.

The 4-East Nursing Staff has been trained in the application of the Bunney-

Hamburg psychosis, depression and mania scale and we have added a scale to measure extrapyramidal side effects (EPS) and sedation. The Brief Psychiatric Rating Scale modified by the NIMH behavior rating scale committee under Dr. D. L. Murphy was adopted for biweekly ratings by our nursing staff and is used weekly by "blind" psychiatrist raters.

Hemodialysis of Schizophrenic Patients

Eight patients have completed a 20-week hemodialysis trial (10 real and 10 sham) in a double-blind evaluation of the potential for this experimental procedure. None of the patients improved. A special cartridge was developed to maintain the double-blind. Patients underwent drug-free testing, including CSF studies, endocrine and psychophysiological testing (Drs. Balow, Schulz, Bunney).

This project stretched the expertise of psychiatrists and nursing staff alike because it required severely ill psychotic patients to remain engaged in the project for up to 6 months without intervention of antipsychotic agents. Mrs. S. Beal and her staff are to be complimented for their collaboration and support. This study was the most difficult we have ever undertaken because of the drug-free management, fistula operation for blood access and clinical management.

We received over 150 requests to participate in this protocol: 23 patients were admitted for evaluation and 11 patients started the protocol. Although our report is completely negative, further double-blind evaluation of hemodialysis at other facilities may be needed to make a definitive statement. At this time there is no evidence that hemodialysis is indicated as a treatment for schizophrenia. Dr. Schulz presented the negative results at the American Psychiatric Association Meeting in May, 1980. Dr. van Kammen was invited to present the data at the Karolinska Institute (St. Görans lecture) in Stockholm, Sweden (April 23, 1980).

Data from studies carried out in collaboration with Dr. Buchsbaum (AER, pain testing), Dr. Gillin (sleep), Dr. Weingartner (cognitive function), Dr. Pickar (beta-endorphin) and Dr. Zahn (psychophysiology) are being analyzed.

Presently we are studying plasmapheresis as a means of testing the role of an "endogenous" toxin or immunoglobulins in schizophrenia.

New Assays and Drugs

In collaboration with the Section on Biochemistry, BPB and the Clinical Psychobiology Branch, new assays for measuring peptides in spinal fluid have been started. We presently have drugs available that are relatively specific for the different neurotransmitter systems that we are studying.

Major Findings: Pharmacological

A. Dopamine Stimulation

d-Amphetamine, an indirect dopamine receptor agonist, has been shown to worsen some acute psychotic symptoms. At the same time, numerous clinical reports of increased social contact and therapeutic effects have appeared in the psychiatric literature over the last 25 years. This, therefore, seems a potentially crucial area of investigation for clarifying the limits and nuances of the dopamine hypothesis. Patients receive i.v. amphetamine and i.v. placebo during the initial test-period. This is repeated during pimozide withdrawal and/or lithium.

A standardized rating system has been developed for quantitating behavioral change before, during and after the infusion interview. Additionally, blood samples are taken regularly during the day to monitor amphetamine blood levels, serum prolactin, norepinephrine and DBH levels. Clinically, schizophrenic patients appear to respond differently to amphetamine than depressed patients, although there is heterogeneity within each group. (Several papers are in preparation or have been submitted.) Strikingly, depressed patients do not become more psychotic; only 18 of 46 schizophrenic patients showed increased psychotic symptoms. However, within this schizophrenic group the more severely psychotic patients may improve, while some patients in early remission may get worse. Preinfusion spinal fluid MHPG correlated significantly with the change in psychosis. Neither chronic pimozide (neuroleptic) pretreatment nor lithium attenuated the behavioral effects of amphetamine. No increased behavioral response was observed following amphetamine administration during the pimozide withdrawal phase (DA receptor supersensitivity). Serum norepinephrine is raised 15 minutes after the acute amphetamine administration. This rise over baseline is not affected by either pimozide or lithium. Serum DBH does not seem to be affected by amphetamine. This suggests that amphetamine affects newly synthesized, rather than stored, norepinephrine. However, there may be a competitive inhibition of DBH by amphetamine. Comparative animal data are not available. Lithium, which is supposed to prevent stimulation-induced release of norepinephrine, had no effect upon the amphetamine-induced rise in norepinephrine. Prolactin levels were increased 25 and 40 min. postamphetamine infusion. This was rather striking since the placebo infusions which took place at the same time of the day--between 8-9 a.m.--showed a slight decrease from the rise of prolactin during sleep as a result of diurnal variation. This is in contrast to the expected decrease in prolactin levels following increased dopamine activity. The increase is hypothesized to be due to stress or activation of serotonin mechanisms in the hypothalamus and pituitary.

B. Dopamine Infusion

In collaboration with Drs. Zis, Gold and Yen, Dr. DeFraites initiated DA infusions to measure prolactin, growth hormone (GH), luteinizing hormone (LH) and follicle stimulating hormone in schizophrenic patients during drug-free neuroleptic-medicated and neuroleptic-withdrawal phases. DA does not cross the blood-brain barrier and acts, presumably, at the DA terminals of the pituitary and median eminence. Preliminary data indicate that schizophrenic patients may have an abnormal LH response and a supersensitive prolactin response to DA in the neuroleptic-withdrawal phase.

C. Dopamine Receptor Blockade and Schizophrenia

(a) Pimozide in acutely psychotic patients - following a washout period, patients are started in a double-blind, placebo-controlled trial of the effects of pimozide in acutely psychotic and remitted patients. This antipsychotic agent is of special interest because of its relatively specific postsynaptic dopamine receptor blocking effect.

During this project endocrine studies and CSF studies are conducted. Selected patients are put on sleep study to evaluate the effects on sleep. In collaboration with Dr. Gillin we have shown that pimozide prevented amphetamine from having any effect on sleep. Rapid eye movement sleep decrease is completely prevented by pretreatment with pimozide. This is in contrast to the behavioral effects of amphetamine, which are reversed in the people who improve while drug-free after amphetamine and who then, after pimozide, become more psychotic. We also gave amphetamine infusions seven days after pimozide discontinuation to evaluate whether receptor hypersensitivity is connected with a change in behavioral response. Data from this project are presently under analysis. However, we did not observe a stronger response to amphetamine, which questions the dopamine hypersensitivity hypothesis of psychosis. A paper evaluating the effects of pimozide on sleep in psychiatric patients has been published, as well as a paper on the pimozide attenuation of the amphetamine-induced REM suppression.

Other studies include endocrine studies, cyclic AMP in CSF, lack of effect of pimozide on DBH in spinal fluid, metabolites of dopamine, norepinephrine (decrease) and serotonin in the spinal fluid and 24-hour urine collections, in addition to the standard battery of behavioral ratings. In collaboration with Dr. Heykants of Beerse, Belgium, we are measuring pimozide levels in our patients with a radioimmunoassay. These levels will be examined in relationship to the above-mentioned hormones and urinary free cortisol. We are now exploring CSF and endocrine variables at different time-points of the pimozide trial, including during the withdrawal phase.

D. Lithium in Schizophrenia

Since the behavioral activation induced by amphetamine also was attenuated by it, lithium may have some antidopamine effects. We studied lithium carbonate in psychotic and remitted schizophrenic patients: nine of 13 psychotic patients responded with an improvement either in psychosis or depression. Diagnostic categories did not separate the responders from the nonresponders. Six of 11 remitted patients who showed postpsychotic depression improved on lithium. We are now studying biochemical and other biological variables to determine which of these may predict a response to lithium. One paper has been published and one paper is in press. Response to d-amphetamine appeared to correlate with antidepressant and antipsychotic response to lithium carbonate treatment for three weeks. This study needs replication.

E. Gamma-Aminobutyric Acid and the Dopamine Hypothesis of Schizophrenia

(a) A hypothesis has been developed about an ineffective feedback system regulating dopamine in psychosis. Gamma-aminobutyric acid (GABA) seems to be implicated in this role. A review paper has been published.

(b) Diazepam, one of the first compounds found to increase GABA function in the brain, was tried on our patients. We administered diazepam in doses higher than 40 mg per day to five patients. Only two severely psychotic patients received 300 mg of diazepam with equivocal results. One patient, who was still relatively nonpsychotic following pimozide withdrawal, became acutely psychotic when he was withdrawn from 70 mg of diazepam. The more psychotic the patient, the fewer the side effects there were like ataxia and sedation. A paper is in progress by Dr. Jimerson.

(c) GABA levels in CSF of schizophrenic patients are presently being studied in collaboration with Drs. Post and Bunney, as well as with Dr. Hare. In 17 schizophrenic patients five GABA levels were lower than in controls, although there was considerable overlap.

F. Endocrinological Project

Endocrinological studies remain a major investment of the Section.

(a) Evaluation of baseline hormonal levels in schizophrenic patients is being conducted, focusing on hormones that are under total or partial control of dopamine. Prolactin, growth hormone, FSH and LH are measured in serum. Measurements thus far have been made with placebo, chronic pimozide, GHB propranolol and naltrexone. Increase in prolactin levels follows dopamine blockade (by pimozide) as expected.

(b) We also examined the growth hormone and prolactin response to apomorphine in drug-free psychotic patients. Patients participated three days after neuroleptic withdrawal, three weeks after placebo and one day after GHB withdrawal. These data are presently under analysis.

(c) Urinary-free cortisol (UFC) levels in drug-free patients and in patients on opiate, pimozide and on the antagonist, naltrexone, are being examined. In contrast to the report by Drs. Chen and Carrol, some schizophrenic patients do seem to have increased levels (like depressed patients). Naltrexone had no effect. Pimozide seemed to normalize UFC levels. During pimozide withdrawal a further significant decrease in UFC was observed (paper in preparation).

(d) Chronic pimozide treatment increases serum prolactin and decreases LH. A paper has been submitted.

G. Cerebrospinal Fluid Studies (CSF) in Schizophrenia

Baseline and probenecid-accumulated metabolites of dopamine, norepinephrine, serotonin, tyramine and phenethylamine in CSF are being measured with mass spectrography.

In 12 patients HVA was evaluated twice during their stay in drug-free conditions--initially, when they were psychotic and four months later when they had improved. During their second drug-free lumbar puncture, HVA appeared to be decreased as was the psychosis rating.

Early evaluation of pimozide's effect upon HVA showed that eight responders had a subsequent rise of HVA but three nonresponders had a decrease. Further patient data have not supported this early observation.

Measurement of viral antibody titers in CSF has been temporarily discontinued.

CSF studies for c-AMP and c-GMP - mean values were not different from depressed comparison groups of patients. Probenecid increased the baseline values. The effects of pimozide are presently under study.

A study of pterine cofactor activity in our patients and in normal comparison groups has started (J.C. Garbutt, R. Levine, W. Lovenberg and J. Ballenger). Schizophrenic patients had similar levels to 20 controls. However, process schizophrenic patients had lower levels. A significant negative correlation with platelet MAO in ten males was observed.

Evaluation of peptides in CSF has been started.

For evaluation of CSF GABA see above; for CSF NE and DBH, see below.

H. Norepinephrine (NE)

(a) Plasma DBH and NE are measured in the serum of our patients while they are drug-free, on lithium and on pimozide. Neither pimozide nor lithium seemed to affect circulating serum levels of both substances.

(b) Dr. Tallman measured β-norepinephrine receptors on leukocytes from psychotic and nonpsychotic patients during drug-free treatment and on d-and dl- high-dose propranolol. There appears to be no difference between schizophrenic patients and normal controls while the receptor count stays stable over time.

(c) DBH in CSF of schizophrenic patients showed similar levels to that of normals but good premorbid (reactive patients) appeared to have lower levels. Dr. Sternberg presented our data at the APA. Patients on pimozide improved in psychosis relative to the decline in CSF NE ($r = .71$, $p < .02$). A paper has been submitted. Dr. van Kammen presented this data at a CINP satellite symposium in Kiruna, Sweden on June 27, 1980.

(d) Dr. Marian Kafka measured α-receptor activity in our drug-free patients. Although the α-receptor activity is not different in schizophrenic patients compared to normal controls, in the male patients c-AMP production was 50% lower than in the controls.

(e) A clinical evaluation of d- and dl-propranolol is under way with concomitant measurement of plasma levels, β-receptor binding, endocrines and CSF metabolites.

I. Endorphins

(a) In collaboration with Drs. Schulz, Wagner, Pickar, Davis and Li, we have reported our studies with β-endorphin infusions to schizophrenic patients for behavioral and endocrinological evaluation. Prolactin was raised by β-endorphin but doses between 10-15 mg did not influence behavior.

(b) A study with a longer acting opiate antagonist, naltrexone, has started. Initial results have been reported by Dr. Davis. Three of five patients improved. Pain-tolerant schizophrenic patients showed reversal of this tolerance with naltrexone.

Major Findings: Physiological - Anatomical

A. Laterality Hypothesis

Lee Mann tests all patients with the Luria test for lateralized sensory and motor function. Data will be compared to drug response and CAT SCAN abnormalities.

B. CAT SCAN abnormalities have been found in about 60% of our patient population, independent of drug use and duration of illness. Psychological and biological exploration of this finding is underway.

C. Positron Emission Scans: This new method of evaluating brain function is ready to be started in drug-free patients.

D. EEG Recording of Sleep

Sleep studies in relationship to amphetamine and pimozide have been undertaken. Pimozide appears to prevent the amphetamine-induced REM suppression (see Pharmacological Findings). Hemodialysis does not seem to affect sleep in our patients.

E. Psychophysiological Testing

(a) Dr. Zahn of the Laboratory of Psychology and Psychopathology is testing our patients during drug-free treatment as well as during different drug conditions.
(b) Pain testing and average evoked response (AER) potential studies are being conducted by Dr. Buchsbaum.

Major Findings: Behavioral and Psychological

A. Drug Withdrawal

We observed that eight of 24 psychotic patients, whom we were not able to differentiate clinically on admission, remitted when their antipsychotic medication was withdrawn over a 30-day period. Although these patients all had been diagnosed schizoaffective-schizophrenic according to RDC classification (1975), this diagnosis did not differentiate the drug-free remitters from the schizoaffective nonremitters. The drug-free improvers appeared to have a later age of onset, a shorter time of hospitalization and better premorbid functioning. The drug-free improved male patients had low platelet MAO activity below the mean for normals, while the nonimproved patients had platelet MAO activity ranging from high to low. The drug-free improvers also responded to lower than the suggested dose ranges for the nondrug-free improvers. This continued to be present at one-year follow-up (150 vs. 484 clorpromazine units/day). This finding may lead to a differential approach of medicating acutely psychotic patients. It suggests that if acutely schizophrenic patients do not improve on neuroleptic drugs at 4-6 weeks, the dose may need to be decreased rather than raised in some patients. The phenomenon of brief improvements during drug withdrawal will be explored further.

B. Hemodialysis

Eight patients have finished 20 dialyses each that included 10 active ones; none of the patients responded and four worsened.

C. Information-processing Project

(a) A simply administered, objective, quantified measure of cognitive disorder has been developed. Preliminary results have disclosed a highly significant difference between patients and normals. We will use this technique to investigate the effects of psychoactive drugs and state variables on schizophrenic thought disorder. We are also investigating the presence and degree of thought disorder in the families of schizophrenic patients which appeared to be present in at least one parent. A paper has been accepted for publication.

(b) Schizophrenic thought disorder is also being investigated with a battery of psychological tests mainly derived from standard I.Q. tests; some scores appear to be impaired in schizophrenia. This emphasizes the need for a special psychological evaluation of our patients.

(c) Evaluation of memory is underway with studies by Dr. Weingartner.

Major Findings: Genetic

A. HL-A Typing Acute Schizophrenia

HL-A antigens are genetically determined human cell membrane antigens found on a variety of cell types. The genes controlling their expression are found on chromosome 6 and are thought to be linked closely to genes controlling immune response. In recent years, associations of certain HL-A types and certain diseases have been found. These diseases are frequently those with associated or causal disorders of immunity, possibly the result of aberrant immune-response genes--thus their association with HL-A.

Schizophrenia may have causal or secondary immune abnormalities; therefore, a study of HL-A antigen frequencies in this disease seemed worthwhile. If found, this would constitute more evidence for the role of the immune system in schizophrenia. To date we have typed 75 schizophrenic patients.

C. Monoamine Oxidase (MAO)

Monoamine oxidase (MAO) in plasma or platelets has been implicated in vulnerability to chronic unremitted schizophrenia and affective disorders. We are presently evaluating clinical and biochemical variables to activity levels. Preliminary data in our male drug-free remitted schizophrenic patients indicate lower than the mean compared to a normal control population. A brief communication has been published. Our patients appear to have significantly lower MAO activity than normal controls.

Significance to Mental Health Research

These studies contribute to the understanding of schizophrenia. Our work integrates the multidisciplinary approaches to this disorder.

Pimozide attenuated the amphetamine-induced sleep changes indicating a role for dopamine in the observed sleep disorder. This finding may have implications for narcolepsy, a disorder in which amphetamine increases wakefulness.

Hemodialysis: The claims of miraculous cures (1977) are under investigation throughout the world. To date (June 1980) there are many places in this country where hemodialysis is already being used as a treatment. Our double-blind evaluation study did not show improvement in our patients. Dialysis for schizophrenic patients should remain an experimental procedure until other double-blind studies indicate definite improvers.

Lithium can be of help in the management of schizophrenic patients, although it appears less effective than neuroleptics. It may have antidepressant properties in postpsychotic depression.

Serum and CSF DHB was lower in some good premorbid, neuroleptic-responsive schizophrenic patients. CSF NE was higher than in normal controls but decreased with neuroleptic-induced improvement. These findings do not support the Stein and Weiss hypothesis of schizophrenia. However, NE correlated with the age of onset in female patients.

Prostaglandin-stimulated cAMP formation is less in schizophrenic males and females compared to normals. This may implicate prostaglandin in schizophrenia.

Schizophrenic patients as a group appeared to be more pain tolerant than normals. This could be reversed by naltrexone, which may imply a disturbance of endorphin regulation of pain mechanism in schizophrenia.

The amphetamine-induced improvement in psychosis and the relative lack of worsening during the pimozide-withdrawal-phase amphetamine infusion raises questions about generalizing the DA hypothesis of schizophrenia and suggests that DA subsensitivity may be present in chronic schizophrenia.

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van Kammen, D.P., Bunney, W.E., Jr.: Heterogeneity in response to amphetamine in schizophrenia: effects of placebo, chronic pimozide and pimozide withdrawal. In: Catecholamines: Basic and Clinical Frontiers. Usdin, E., Kopin, I.J., Barchas, J. (Eds.), Oxford, Pergamon Press, 1979, pp. 1896-1898.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00081-06 BP

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Heritable Characteristics of Cation Transport in Primary Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	D. C. Jimerson, M.D.	Staff Psychiatrist	BP	NIMH
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COOPERATING UNITS (if any)

Illinois State Psychiatric Institute, Chicago, Illinois; University of Chicago

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is the identification of heritable characteristics of ion transport which may distinguish individuals with a primary affective disorder from normal controls.

A study of sodium potassium stimulated adenosine triphosphatase (Na^+-K^+-ATPase), a cation transport enzyme, is in progress. The level of this membrane enzyme is lowered in depressed patients but returns to normal during remission. A twin study will serve to determine heritability of this enzyme. We are also looking at effects of amphetamine and arecoline on this enzyme.

New results from other centers have led us to reopen our interest in lithium transport. We are now engaged in a collaborative program of research on the lithium erythrocyte/plasma ratio in euthymic patients. We have confirmed that Bipolar patients have higher ratios than controls. We propose to investigate this ratio in relatives of patients to test whether it may be a genetic vulnerability factor in affective disorder.

PHS-6040
(Rev. 10-76)

Project Description

A spectrophotometric assay has been used to determine levels of $Na^+ - K^+$ - ATPase in human erythrocytes. Values in normal controls have been found to be not significantly different from those of patients with affective disorder in remission in two separate studies. Patients who are in euthymic or hypomanic states have significantly greater enzyme activity than those who are depressed ($p < .05$), during lithium administration.

We propose to study this enzyme in monozygotic twins to assess its heritability. We also propose to examine response of the enzyme to arecoline and amphetamine stimulation, since arecoline has induced depressive symptoms and amphetamine may induce excitement or depressive symptoms in patients with affective disorder.

Lithium transport parameters have been extensively studied by Pandey and colleagues at the Illinois State Psychiatric Institute. They reported lithium erythrocyte/plasma ratio to distinguish between ill and well relatives within pedigrees and thus proposed it to be a genetic vulnerability factor for affective disorder. We have entered into a collaboration with them in an attempt to replicate this finding on our population of patients with affective disorder and their relatives. Preliminary results confirm that patients have higher ratios than controls ($p < .02$). Questions that are now being pursued include the reliability of the measurement and its relationship to time off lithium (the assay may be done in vitro or in vivo). If satisfactory answers to these questions are obtained, we will proceed to a study of relatives of patients.

Forty-eight patients and 10 normal volunteers participated in these studies.

Significance to Mental Health Research

Cation transport mechanisms depend on cell membrane characteristics that are likely to be largely genetically determined. Previous studies have demonstrated differences in cation transport mechanisms in affectively ill patients. We have confirmed differences in Na^+-K^+-ATPase during depression but not in the well state. Thus it is unlikely to be a trait marker but may be useful as an indicator of state changes. As part of a pharmacologic challenge strategy, it may also be informative in differentiating patients from controls.

The finding that lithium erythrocyte/plasma ratio acts as a genetic vulnerability factor in pedigrees of people with affective disorder is extremely important to confirm or disconfirm. Such a factor would be important in predicting illness within families as well as understanding the etiology of affective disorder.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00082-06 BP

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Life Events, Family Attitudes, and Marital Dysfunction in Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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0.7	0.4	0.3

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the investigation of life events is to measure the relationship between the number and kind of life events - the amount of subjectively felt social support and social alienation to mood disorders in affectively disturbed patients versus their normal relatives who are used as controls. A new scale, the Family Attitudes Questionnaire (FAQ), was developed to measure patients' and relatives' perceptions of the morbid risk for depression or mania or schizophrenia, of the burden these disorders create for the family, and the effect this may have on future plans of a family member for marriage or childbearing. In Bipolar illness, 19 married patient/spouse couples, where marriages had been stable for at least two years, responded to the FAQ. Fifty-three percent of the spouses said they would not have married knowing of the disorder but none of the patients did. Fifty-eight percent of the spouses said they would not have had children knowing of the possible risk of transmission. In schizophrenia, patients rated burden and risk lower than their parents, but well sibs gave lowest risk/burden estimates.

I. A Dysfunctional Attitudes Scale was given to a sample of 32 out-patient manic depressive patients and their spouses. No significant difference was found between patient and spouse attitudes. The scale is currently being given to a group of normal controls.

Objectives:

I. Life Events:
  1. To collect standardized data on life events, social support and social alienation.
  2. To compare the data from a group of patients with that from their normal relatives used as controls.
  3. To assess whether there may be a "family culture" of life events which occur among family members.
  4. To correlate the amount of social support perceived by patients and controls with the frequency of life events.
  6. To compare the degree of social alienation between patients and controls.

II. Family Attitudes Questionnaire
  1. To compare the attitudes of patients who are married, who never married, who are separated or divorced with each other and with spouses, ex-spouses and normal siblings toward a) the most troublesome characteristics of the acute stages of mania and depression or schizophrenia, and b) the ongoing social problems caused by these disorders, and their perception of a) the causes for the disorders, b) the degree of risk in the general population and c) the burden these disorders cause in comparison to various medical conditions.
  2. To assess these patients' and relatives' attitudes toward marriage and childbearing as an adjunct to therapeutic intervention or for genetic counseling.

III. Dysfunctional Attitude Scale
  1. To collect data from patients with manic depressive illness and their "well" spouses about their attitudes toward themselves, the future and the world.
  2. To assess the differences/similarities in attitudes of patients and spouses as a test of clinical observations regarding spouse contributions to marital interaction and family dysfunction associated with ongoing social problems related to clinical mood episodes.
  3. To compare patient/spouse's attitudes to a sample of normal controls.

Method:

The Life Events Scale is a self-administered instrument completed by the patient and first degree relatives. The Family Attitudes Questionnaire, initially used as an open-ended interview, was tested for self-administration and subsequently used that way. The Dysfunctional Attitudes Scale is a self-administered scale.

These scales are being given to current and former NIMH patients with affective disorders or schizophrenia and their families, and to normal controls recruited as a comparison family study sample. We are currently investigating family attitudes in divorced and never-married patients, ex-spouses, and siblings.

Progress to Date:

The Life Events, Social Support and Alienation Scale has been completed by 57 manic-depressive patients and approximately 200 first degree relatives of patients with this disorder. These data are now in the process of being analyzed. Thirty-four of the patients were hospitalized at the time of completion of the scale and 23 were in remission. These groups will be compared for the number and kind of events. The LES scores of first degree relatives, diagnosed as normal in personal interviews, will be compared to both samples. We are interested in learning whether certain events or whether the number of events are related to episodes of illness.

The Family Attitudes Questionnaire has been completed by the original 19 patients and their spouses, plus 21 never-married patients, 21 separated or divorced patients, and 16 normal siblings of patients. We have also been successful in receiving responses from 10 ex-spouses of patients, a group which is very difficult to find. This data is ready for coding and analysis.

The Family Attitudes Questionnaire was also used with 17 hospitalized schizophrenic patients when they were ready for discharge and 27 of their first degree relatives. The results of this sample showed a disparity between the attitude of well family members and patients. Ninety-two percent of the parents compared to 25% of the patients identified schizophrenia as a disorder associated with extreme burden (p .001). Only 29% of the parents compared to 66% of the patients said they would have children based upon what they now know about schizophrenia in their family (p .05). However, 87% of the well siblings said they would have children knowing about the disorder in the family. Patients and their siblings therefore differ from their parents on childbearing, perhaps because the desire to have children overrides considerations of risk and burden.

The Weissman Dysfunctional Attitudes Scale has been used by 32 euthymic outpatients and their spouses to determine spouse's contribution to ongoing family dysfunction in the marital interaction of manic-depressive patients and their partners. No significant differences were found between the patients' and the spouses' scores. These scores do not differ significantly from the scores of a normal population reported in the literature. It would appear that the "dysfunctional attitudes" as measured by this scale are not depressogenic as claimed. However, to test this further we are using the Scale with our own sample who have been diagnosed as normal by our standardized criteria. To date we have a control sample of 23. Our interest is in trying to contribute to the theoretical understanding of marital interaction in marital pairs in which one partner has the disorder. The chaos and ongoing stress found in these families has traditionally been attributed to the mood disorder. It is our clinical observation that the partner's attitude or perception may also be contributing. Therefore it may be important that the partner, as well as the patient, needs to be included in therapeutic planning.

Proposed Course of Project:

Data collection will be completed for both the Family Attitudes Questionnaire and the Dysfunctional Attitudes sample and prepared for analysis and reporting. The Life Events Scale is being processed for analysis and reporting.

Significance to Mental Health Research:

The family culture of life events, or of families in which a member has manic-depressive illness, has not been studied, to our knowledge. The attitudes and perceptions held by these families may be transmitted from generation to generation as much as biologic genetic components may be. It is important to investigate what these attitudes are so that appropriate therapeutic intervention may interrupt whatever influence they might have on future generations. In this sense, intervention may be viewed as preventive.

Both the Family Attitudes Questionnaire and the Dysfunctional Attitudes Scale have been shown to be useful in supplementing clinical observations in the families who are receiving outpatient treatment or in premarital and pre-childbearing counseling with couples requesting it.

Publications:

1. Targum, S. D., Dibble, E. D., Davenport, Y. B., and Gershon, E. S.: The family attitude questionnaire: Patients and spouses view Bipolar illness. Arch. Gen. Psychiatry. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00083-06 BP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Hypothesis of X-linkage in Bipolar and Unipolar Affective Disorder

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. McGinniss	Research Biologist	BB	CC

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0.0	0.0	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been completed.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00084-06 BP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Genetic-Biologic Studies of Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3.8	2.0	1.7

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Family study of primary affective disorders has reached the stage of completed data collection. Schizoaffective patients have the highest proportion of illness in relatives, followed by Bipolar patients (types I and II), and then Unipolar patients. A significant rate of illness in spouses of patients, implying assortative mating, is present. Genetic analyses applied to these pedigrees have failed to identify a homogeneous sub-group with a common mode of transmission. Theoretical studies of the power of single locus pedigree analysis methods using simulation techniques are ongoing. Analysis of genetic transmission of activity of enzymes of catecholamine metabolism revealed codominant autosomal single locus transmission as most likely for both catechol-O-methyltransferase (COMT) and dopamine-β-hydroxylase (DBH), whereas monoamine oxidase activity was not well accounted for by either X-chromosome or single locus transmission. Cell culture studies reveal a genetic component in aging processes at the cellular level, which appear to be of general significance in the aging process. Genetic analyses of patients in whom cerebrospinal fluid (CSF) metabolites have been studied revealed that

Summary (Continued)

patients with positive family history of alcoholism or sociopathy have significantly lower baseline levels of homovanillic acid (HVA), 3-methoxy-4-hydroxyphenyglycol (MHPG), and norepinephrine (NE).

Project Description

1. Family study of affective illness - Data collection and purification are now complete. This process, including laborious retrieval of medical records from numerous sources and weeding out of patients who did not have primary affective disorder with no diagnostic complicating factors, has taken well into the current fiscal year. Nearly one-half of the patients originally admitted to NIMH wards were discarded for reasons of diagnostic questions or unavailability at the time of attempted follow up. At this point there remain 31 Unipolar, 94 Bipolar I, and 36 Bipolar II probands in the sample. In addition, several probands originally thought to be Bipolar were found to be schizo-affective upon rigorous application of diagnostic criteria. We have included statistics on 4 of them (randomly chosen) for a comparison in the present figures.

TABLE 1

Lifetime History of Psychiatric Illness in 913 Parents, Siblings, Adult Offspring

	Patient Diagnosis			
Relatives' Diagnosis (%)	S-A	UP	BP I	BP II
Bipolar I (BP I)	11	1	5	2
Bipolar (BP II)	4	1	4	4
Schizo-affective (S-A)	4	1	1	1
Unipolar (UP)	11	12	11	14
Total Major Affective	30	15	21	21
Minor Depression	0	9	5	4
Cyclothymic	0	0	3	3
Alcoholism	7	7	4	4
Drug Abuse	4	1	1	1
Total Minor Affective	11	17	13	12
No Discernible Diagnosis	43	47	52	52

If we look on genetic virulence as expressed by the proportion of relatives with major affective illnesses, then schizo-affective disorder would appear to be the most virulent and Unipolar depressive disorder (no history of mania or schizophrenic symptoms) the least virulent. As in other studies, the relatives of schizo-affective patients tend to have affective disorders and schizo-affective disorder but not schizophrenia. Applying the rigorously developed diagnostic criteria tested in a previous population study, we note that 21% of the relatives of Bipolar I or Bipolar II patients have major affective illness, as do the somewhat smaller proportion of 15% of relatives of Unipolar patients. As in other studies, the Unipolar patients have relative deficit (but not complete absence) of Bipolar illness in their relatives. This again is compatible with the hypothesis of a continuum of genetic virulence in these disorders with Unipolar illness being the least virulent.

The prevalence of major affective illness in the relatives of these patients is higher by a factor of approximately 5 than the prevalences in a previously diagnosed population sample. The prevalence in relatives of nonpsychiatrically ill medical patients of the National Institutes of Health is currently being studied, and final results are not yet available. Similarly, population prevalences from our collaborators at Yale University are also not yet available, and we estimate that both sets of prevalences will become available only about a year from now.

The rate of illness in second degree relatives (grandparents, uncles and aunts, and half-siblings) is considerably lower than that in first degree relatives. For example, 7% of grandparents, uncles and aunts, and 6% of half-sibs, have major affective disorder. This still appears to be higher than the population prevalence, which is not greater than 3 1/2% according to existing population data using the same diagnostic methods.

The rate of psychiatric illness in 140 spouses of patients with affective disorders appears to be elevated: 7% of spouses of Bipolar I patients, 11% of spouses of Bipolar II and 8% of spouses of Unipolar patients have a major affective disorder. This confirmation of assortative mating is of some interest, as is the finding of no Bipolar I spouses of Biopolar I patients. This suggests that such alliances are extremely unstable.

The issue of sex chromosome transmission in Bipolar illness has been repeatedly raised. One test for sex chromosome transmission is to look for absence of male to male transmission, expressed as very low prevalence of affective illness in fathers and sons as opposed to mothers and daughters of male Bipolar patients. We find 14% of 81 fathers and sons of Bipolar patients to have major affective illness, as opposed to 20% of 76 mothers and daughters. This suggests that such linkage is not present.

2. Genetic Analysis of Affective Disorders.

On the basis of initial analyses on a group of 15 of the largest pedigrees in our sample (approximately 500 informative individuals), no single gene autosomal hypothesis of transmission of illness could be shown to fit the data. In attempts to resolve possible genetic heterogeneity among families with affective disorders, a sample of 18 pedigrees (approximately 450 informative individuals) was examined for single gene transmission of illness. The probands of these families had primary Bipolar illness with an age of onset before 30 years. As in the mixed sample, no autosomal single gene hypothesis fit the data and a strictly non-genetic hypothesis was also excluded. We can conclude that at least in our sample, early age of onset does not define a subtype of affective disorder determined by a single major locus.

Further analyses will use re-definition of diagnostic criteria according to interview information and biological measures already on file, to search for single-gene compatible transmission.

Theoretical studies of the power of the single locus methods to detect a single gene in the presence of varying probability of gene expression (penetrance) and possible genetic heterogeneity are ongoing. In collaboration with Dr. Kenneth K. Kidd at Yale University, multigenerational pedigrees have been simulated under various single gene models of transmission of an illness. Generally, we find that autosomal transmission is correctly detected if sample size is large enough (between 250-750 relatives depending on family structure and genetic model being studied). However, gene frequency is consistently overestimated. The power of detecting the major locus and the accuracy of parameter estimates are best when penetrance is high or intermediate (40-70% for heterozygotes). If heterozygote penetrance is low (5-15%, as in recessive traits) parameters estimates were not consistently accurate, and there was not always clear discrimination between Mendelian and non-genetic hypotheses. A major gene could be detected in the presence of a high precentage of phenylcopies (non-genetic illness) if the sample size was large enough.

We have recently added to these autosomal analytic methods single locus X-chromosome pedigree analytic methods, and the power of discriminating X-chromosome and autosomal transmission in the same data set will now be tested on the simulated data. We are also looking into sex-specific parameters as they affect transmission of illness.

These studies are required because the basis of use of these analytic methods is entirely theoretical, and their actual power in data analysis can only be tested through these simulations.

3. Genetic transmission of enzymes of catecholamine metabolism (DBH, COMT, and MAO)

Activity of dopamine-β-hydroxylase (DBH), erythrocyte catechol-O-methyltransferase (COMT), and monoamine oxidase (MAO) were compared in various groups of patients, normal relatives, and normal controls. DBH and COMT did not differ in any of these groups. MAO was lower in patients than normal controls but normal relatives also had lower values than controls. We concluded that none of these enzymes are markers of genetic vulnerability to affective disorders. However, our extensive pedigree data allowed us to study the genetic transmission of DBH, COMT, and MAO activity.

For all three enzymes, variation among families was significantly greater than variation within families indicating that familial factors affect ther activity. To analyze the familial pattern more specfically, single gene autosomal and X-chromosome hypotheses were tested using the segregation analysis method of Elston and Stewart. For both COMT and DBH, a purely non-genetic hypothesis could be rejected. In addition, autosomal dominant and recessive gene transmission were also rejected. However, codominant genetic transmission could not be rejected. Under this hypothesis, heterozygote individuals are distinguishable from homozygotes. This finding implies that the distribution of COMT and DBH values in our sample actually consist of three underlying distributions, one for each of the three genotypes. In this sample, the single gene accounts for approximately 50% of the variance. For COMT, autosomal codominant single gene transmission was corroborated by Weinshilboum's finding of thermolability in the homozygotes for low activity. Hypotheses of X-chromosome transmission could not be strictly rejected, but they were much less likely than autosomal hypotheses.

In the analysis of MAO we found that both X-chromosome and autosomal single gene hypotheses were compatible with the data, both hypotheses having a nearly equal likelihood. The X-chromosome transmission hypothesis predicts a dominant allele for very high MAO that accounts for approximately 11% of the variance. The autosomal hypothesis predicts a codominant allele that accounts for 25% of the total variance. These hypotheses are not mutally exclusive -- in theory both X-chromosome and autosomal genes could affect MAO activity. However, even though both of these hypotheses are compatible with the data, neither one accounts for a very large proportion of the total MAO variation. It is possible that the majority of the variation is due to other polygenic and/or environmental factors. Analysis of the data with a mixed model of inheritance (i.e. a model that allows for tests of both polygenic and major locus hypotheses) may resolve this uncertainty. We are currently setting up this analytic method.

4. Cell culture

We have continued our cell culture studies with our collaborators in Chicago and New Haven. A genetic component in the life span kinetics of cells in culture is strongly suggested by differences in cell proliferation and replicative life span. This suggests genetic components in the aging processes at the cellular level, and appears to be of general significance in the aging process.

5. CSF metabolites in relationship to family history of psychiatric illness

In an ongoing collaboration with Drs. Post and Goodwin, we have been studying the relationship of CSF metabolite levels to family history of affective diagnoses in patients with major primary affective disorder. CSF data are available on approximately 60 patients for whom we have extensive family study data.

In preliminary analyses, after correction of metabolites for age, sex, weight, and probenecid level, we have examined differences in metabolite levels (5-HIAA, HVA, MHPG, and NE) according to the presence or absence of specific diagnoses in their first degree relatives. Patients with a positive family history of alcoholism or sociopathy (corresponding to Winokur's definition of depression spectrum disease) have significantly lower baseline levels of HVA, MHPG, and NE. No differences in post-probenecid CSF metabolite levels were found. Patients with a positive family history of major primary or secondary affective disorder did not differ in any of these metabolites from patients without such a family history.

Using the opposite approach, patients were divided into two groups ("low" and "high") according to their metabolite levels. The frequency of primary major affective disorder in first degree relatives was then compared between the "low" and "high" groups with respect to each CSF metabolite. No significant differences were found.

Further analyses will attempt to clarify the findings in relation to alcoholism and the apparent absence of metabolite findings in relationship to family history of affective illness.

6. Conference on Genetic Research Strategies in Psychobiology and Psychiatry

A conference chaired by Dr. Gershon and supported by the National Institute of Mental Health and the Foundations' Fund for Research in Psychiatry was held in Florida in December, 1979, as a satellite to the American College of Neuropsychopharmacology Meeting. At this conference, a broad consensus was reached on promising strategies for new research. Biological genetic studies in general, and studies using cell culture and recombinant DNA techniques in particular, seem to be particularly opportune for psychiatric and psychobiologic genetics at this time. In connection with this, we are attempting to establish a small cell culture laboratory in the Section, and to bring on a senior scientist who can set up recombinant DNA studies of peptides and other large molecules involved in psychobiological characteristics.

Future Course of Project

In the immediate future, the cell culture laboratory and laboratory for molecular genetic studies have the highest priority.

We expect to continue our mathematical studies of genetic modeling as applied to psychiatric diagnoses and biological and pharmacological characteristics of patients and relatives. Further studies of diagnosis in relatives of patients with affective disorders, particularly children, are planned. Our ongoing project on relatives of patients with anorexia nervosa, and relatives of patients with medical disorders, should be completed in about 12-18 months.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00085-04 BP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Pharmacogenetics of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.8	1.4	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The inheritance of variability in the behavioral and physiologic effects of d-amphetamine is being studied. A small dose of amphetamine (0.3 mgm/kg) was administered to normal volunteer twins after medical and psychiatric screening. Identical twins are highly concordant in behavioral responses, growth hormone and prolactin changes, measures of sleeplessness and attention, and baseline 3-methoxy-4-hydroxyphenylglycol (MHPG). Behavioral response to amphetamine is highly predictable from baseline measures of MHPG and prolactin.

A cholinergic agonist, arecoline, has been used to test the REM-induction response in identical twins. Preliminary results indicate that this response is not heritable.

Cortisol response to arecoline has also been tested in normal volunteers, where a threshold dose for plasma cortisol increase has been identified.

Other Investigators Continued:

J. C. Gillin, M.D.	Chief, Unit on Sleep Studies	BP	NIMH
D. C. Jimerson	Staff Psychiatrist	BP	NIMH

Project Description:

Pharmacogenetic studies in psychopharmacology have largely been devoted to inherited differences in drug metabolism as opposed to genetics of physiologic and psychologic responses to drugs. The behavioral pharmacogenetics of psychoactive drugs in particular is an area in which little systematic investigation has been undertaken, although there have been enough clinical reports of patients and relatives responding to the same medication to suggest that further study will be fruitful.

Our first pharmacogenetic protocol was a study of d-amphetamine. Individual differences in response to amphetamine have been used as a predictor of clinical response to tricyclic antidepressants and as such may be an important clue to the genetic vulnerability to affective disorders. In this project, monozygotic and dizygotic twin volunteers were studied. All persons with pre-existing psychiatric or medical disorders that are not minor were excluded.

d-Amphetamine is an indirect agonist of dopamine and norepinephrine neurons in the central nervous system. There has been much recent interest in the effects of amphetamine and amphetamine-like compounds such as methylphenidate in mania, schizophrenia and depression.

Amphetamine may produce mood changes of excitation or depression. Results on thirteen monozygotic twin pairs and three dizygotic twin pairs show that these responses are likely to be heritable (intraclass correlation for the excitation response was 0.70 and for the depression response was 0.58). The excitation response was found to be more reproducible within an individual than the depressive response. Plasma amphetamine half-lives were also found to be reproducible in an individual and heritable. The behavioral responses were not correlated with amphetamine levels, however, suggesting heritable differences in drug response as well as drug metabolism. An elation response was also correlated in identical twins (intraclass correlation 0.47) and was reproducible. This response was, however, directly related to amphetamine level.

Use of the patient activity monitor (PAM) indicated that the amount of nocturnal activity following amphetamine was heritable and it was stable in individuals over time.

Serum prolactin was found to show wide individual differences in response to amphetamine. These responses also seemed to be stable and heritable; the normal individuals we studied had a mean decrease in prolactin as opposed to the increase noted in schizophrenic patients in another NIMH study. Growth hormone response was also stable and heritable. This is important because of reports that this response is blunted in depressed patients. Results of our study of patients should indicate whether this finding remains during remission.

Averaged evoked responses (AER) have also been measured following amphetamine. Attentional enhancement by amphetamine was found to be highly concordant in identical twins (intraclass correlation 69).

Factor analysis showed that a grouping of variables including high baseline MHPG, high growth hormone, and high excitation score was highly correlated in MZ twins. There is evidence from other studies that these variables are related to norepinephorine activity. We hypothesize that this factor may result from heritable differences in norepinephrine receptors and we plan to test this by using specific norepinephrine blocking agents along with amphetamine.

We have also tested amphetamine response in patients with primary affective disorder in remission (see report on project number Z01 MH 00086-04 BP).

We have also investigated heritability of the REM sleep induction response to arecoline, a cholinergic agonist. This response has been found to differentiate affectively ill patients in the well state from controls. We have tested seven pairs of monozygotic twins; two pairs are discordant with regard to this response.

Thus this response is probably not a genetic factor in the vulnerability to affective disorder. There is some indication from further interviewing of the twins after the study, that marijuana use may have contributed to the discordance. The effect of marijuana on arecoline responses is of interest since other investigators have found that similar combinations have produced depressive symptoms in normal volunteers. We plan to investigate this combined effect further.

Neuroendocrine Responses to Arecoline. One strategy presently under investigation is measurement of plasma cortisol and prolactin as indicators of sensitivity to challenge with the cholinergic agonist arecoline. To date we have administered graduated doses of arecoline to healthy volunteers and have assayed plasma cortisol response. We have established a dose-response relationship, with a threshold dose of approximately 4 mg arecoline subcutaneously. Future plans call for comparision of the cortisol and prolactin response, as well as possible behavioral effects, in Bipolar patients when drug free in the well state, and in age and sex matched controls.

Proposed Course of Project:

We plan to compare results of the amphetamine pharmacogenetic patients. If the technique seems promising for differentiating people at risk for affective disorder, we propose to investigate amphetamine response in relatives of clinic patients (protocol amendment to be written). We propose to test four more pairs of dizygotic twins in this study to compare with our results on monozygotes. We also propose to recall several pairs of twins with a pronounced response on the hypothesized noradrenergic factor in order to test the results of receptor blockade. We also propose to test the combination of arecoline and marijuana in normal volunteers.

Significance:

We have noted heritable differences in response to amphetamine in behavior, evoked responses, hormone levels, and MHPG. If these responses differentiate patients with affective illness from controls, they may be of use in tracing vulnerability to mood disorder through families, and in genetic counseling of family members. This is the first report of pharmacogenetic variation in an affective response in man; it may relate to inherited differences in brain receptors.

Nongenetic variables appear to be responsible for the arecoline REM-induction response. It may not appear productive to pursue this as a possible genetic marker in affective disorder.

Publications:

1. Gershon, E. S., Nurnberger, J. I., Sitaram, N. and Gillin, C.: Pharmacogenetics and the pharmacologic challenge strategy in clinical research. Studies of d-amphetamine and arecoline. In Saletu, B. et al (Eds.), Neuro-psychopharmacology. New York, Pergamon Press, 1979, pp. 75-83.
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00086-04 BP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. Costa, M.D.	Staff Psychiatrist	CN	NIMH

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1.5	1.3	0.2

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The outpatient clinic provides a "well state" patient population for genetic and pharmacologic studies of persons with Unipolar (major depressive) and Bipolar (manic-depressive) disorders. Such a "well state" population is crucial in identifying biochemical, pharmacologic, or physiologic abnormalities which are present in persons vulnerable to affective disorder, and which may serve as genetic markers to affective illness.

Studies of cholinergic sensitivity in Bipolar patients and relatives are continuing. We have confirmed increased susceptibility to arecoline rapid-eye movement sleep (REM) induction in patients never medicated or off medications for months or years. Pupillometry studies show increased sensitivity to pilocarpine in euthymic patients off medications as compared with controls.

Our study of amphetamine response in patients is complete and the data are being analyzed.

(Continued)

OTHER INVESTIGATORS:

D. Murphy, M.D.	Chief	CN	NIMH
E. Silberman, M.D.	Staff Psychiatrist	BP	NIMH

Summary (Continued)

Lithium accumulation in platelets is being studied in collaboration with the Clinical Neuropharmacology Branch of NIMH. Preliminary results indicate that lithium attaches to the dense bodies in the platelet.

A study of the phenomenology of affective illness has focused on seizure-like phenomena during mania and depression. Thirty-five clinic patients have been interviewed in conjunction with this study. Their histories are being compared with groups of epileptics and hypertensives.

Project Description:

The purpose of the project is to have a "well state" patient population available for participation in the following research areas:

1. Identification of biochemical, pharmacologic, or physiologic abnormalities which are present in persons who have had major affective disorder but who do not currently require hospitalization.

2. Identification of biological markers of a genetically transmitted predisposition to affective disorders.

3. Creation of a pool of available patients and relatives for further protocols which will be developed and submitted on the genetics of affective disorders, and on longitudinal studies of affective disorders.

Patients are admitted to the study upon referral from the wards of the NIMH intramural program or from various community referral resources. Sixty-six patients (37 Bipolar I, 17 Bipolar II, 9 Unipolar, 3 others), are currently enrolled in the BPB portion of the outpatient clinic.

Identification of biochemical, pharmacologic or physiologic vulnerabilities to affective illness: The strategy in this type of study is to identify a biologic vulnerability to illness which is not state dependent but present or evocable even when the patient is in the "well state" or in remission.

We have described the results of our twin study of amphetamine response (Z01 MH 00085-04 BP). Amphetamine response in patients has been studied using a similar paradigm. Behavior, growth hormone and prolactin response, biochemical measures such as plasma MHPG and beta-endorphin, and evoked responses are now being analyzed. Eleven clinic patients have participated in this project. If patients are found to differ from normal twins, we will proceed to test responses in ill and well relatives.

Forty-eight clinic patients participated in studies of lithium transport and $Na^+ - K^+$ ATPase (see Z01 MH 00081-06 BP).

Our studies of cholinergic responses in patients have also proceeded. Well state patients who had never been on medications or who had been off all medications for months or years were found to have the same quick REM-induction response to arecoline as those recently on medications. Pupillary response to the cholinergic agonist pilocarpine is more sensitive in remitted Bipolar patients than in normal controls, as found in our collaboration with Dr. N. Sitaram. Arecoline-induced decreases in temperature were tested in six patients and found to be no different than control decreases.

Genetic studies of arecoline sleep induction have been slow to start, but one ill relative of a patient has been studied. This Unipolar mother of a Bipolar patient did not show increased sensitivity to arecoline REM induction.

Dr. John Costa is studying lithium accumulation in the platelet using nuclear magnetic resonance spectroscopy. Seven clinic patients have provided specimens, enabling Dr. Costa to discover that lithium associates with the dense bodies in platelets. These studies may provide information about the molecular mechanism of lithium.

Plasma norepinephrine (NE) metabolites. We have been exploring the possible use of plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) as a marker for pharmacogenetic responses in affective illness. The section has supported continuing refinement of a sensitive and specific gas chromatographic-mass spectrometric assay for plasma MHPG and the precursor metabolite 3,4 dihydroxyphenylglycol. In an initial study we have demonstrated the heritability of free and conjugated levels of MHPG in monozygotic twins. In collaboration with others we have shown a decrease in levels of the metabolite following pharmacologic challenge with the α_2 adrenergic agonist clonidine. Also in collaboration with others we have studied the plasma MHPG responses to alpha methylparatyrosine, monoamine oxidase inhibitors, and naloxone.

In the future, after further analyzing the data in the challenge strategies noted above, we plan to follow the MHPG response to an adrenergic agonist or blocker as a possible marker of alterations in noradrenergic receptor function in patients with affective illness.

Dr. Edward Silberman has interviewed 35 patients in the clinic as part of a study of sensory phenomena experienced in conjunction with seizures. Preliminary results indicate that about a third of affective patients experience such phenomena. This may indicate that a subgroup of affective patients have an illness with some similarities to epilepsy.

In collaboration with Dr. Ellen Frank of the University of Pittsburgh, we have collected data on marital relationships in Bipolar affective disorder. Although some of the same regrets noted in our earlier studies were replicated in this one, the overall marital relationship seems to be about as stable and rewarding in Bipolar patients between episodes as in a control group. This suggests that the episodes of illness rather than chronic personality factors are responsible for the very high divorce rate in Bipolar affective illness.

Dr. Steven Shama interviewed all patients in the clinic and examined many for evidence of acne. Beginning or worsening of acne occurs in about 10-20% of patients after starting lithium treatment (this data is being analyzed). Association of acne with lowered zinc levels has been reported by other investigators. We are analyzing data now regarding zinc levels in our population.

Dr. Cass Connelly has studied compliance with lithium treatment and appointment keeping in our clinic. Compliance was found to be higher in married patients and those who perceived relative continuity of care in their treatment. Compliance was poorer in hypomanic patients. Poor compliance was significantly associated with poor outcome over a 15-month follow-up period.

Planned Clinical Treatment Protocols

Depression in Bipolar patients is treated with conventional antidepressant drugs, episodes of mania and hypomania may be provoked and cycles of mania and depression may be induced. We are undertaking to study various antidepressant treatments in Bipolar patients looking for a treatment which will not induce mania as compared to treatment with lithium alone, but which is still an effective antidepressant. Based on hypotheses about adrenergic induction of mania, we are attempting treatment with an anticholinergic agent (tryhexiphenyldil), agents with predominant serotonergic activity, and β-adrenergic agonists. These protocols are in the planning or institute review board stage now and we hope to begin the studies over the next year.

Significance of the Study:

Identification of a biologic vulnerability detectable in the well state in Bipolar manic depressive illness would be of great importance for clinical treatment and also for identifying persons who might become ill in the future, including relatives of patients. Clinical and longitudinal studies of affective illness are important in increasing our knowledge of the course and treatment of this disorder.

Proposed Course of Project:

Studies of CNS and peripheral sensitivity to agonists and antagonists of monoamine and cholinergic receptors will continue to have highest priority over the coming years.

The use of the clinic as a resource for the study of the clinical and biochemical characteristics of patients with affective disorder has been productive and will continue.

Publications:

1. Sitaram, N., Nurnberger, J. I., Jr., Gershon, E. S., and Gillin J. C.: Faster cholinergic REM sleep induction in euthymic patients with primary affective illness. Science 208: 200-202, 1980.

2. Gershon, E. S. and Hamovit, J. R.: Genetic methods and preventive psychiatry. Prog. Neuro-Psychopharmacol. 3: 565-573, 1979.

3. Nurnberger, J. I., Gershon, E. S., Murphy, D. L., Buchsbaum, M. S., Goodwin, F. K., Post, R. M., Lake, C. R., Guroff, J. J. and McGinniss, M. H.: Biological and clinical predictors of lithium response in depression. In Gershon, S., Kline, N. S. and Schou, M. (Eds.): International Lithium Conferences: Controversies and Unresolved Issues. New Jersey, Excerpta Medica, ICS Series, 1979, pp. 241-251.

4. Gershon, E. S.: New approaches in clinical studies. In Gershon, E. S., Matthysse, S., Ciaranello, R. D. and Breakefield, X. O. (Eds.): Genetic Strategies in Psychobiology and Psychiatry. Pacific Grove, CA., Boxwood Press. In press.

5. Gershon, E. S.: Genetic factors from a clinical perspective. In Mendels, J. (Ed.): Psychobiology of Depression. New York, Scientific and Medical Publishing Co. In press.

6. Targum, S. D. and Gershon, E. S.: Pregnancy, genetic counseling, and the major psychiatric disorders. In Schulman, J. and Simpson, J. L. (Eds.): Genetic Diseases and Pregnancy. New York, Academic Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00117-05 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

α- and β-Adrenergic and Prostaglandin Receptors in Human Blood Elements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian S. Kafka	Physiologist	BP NIMH
	John F. Tallman	Biologist	BP NIMH
Other:	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH

COOPERATING UNITS (if any)
Section on Psychobiology, BPB, NIMH; Section on Neuropsychopharmacology, BPB, NIMH; Laboratory of Clinical Psychopharmacology, NIMH; Clinical Psychobiology Branch, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alpha- and β-adrenergic receptors and prostaglandin receptors are being studied in human blood cell preparations. The ability of adrenergic agonists to activate or inhibit adenylate cyclase is used as a measure of their biological function. Binding of tritiated adrenergic receptor antagonists to membrane receptors on the blood cells is being measured. These correlative measures are used to assess α- and β-adrenergic physiological function in normal human beings, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents.

Objectives:

(1) To develop methods to study the physiological function of α- and β-adrenergic receptors in easily obtainable human tissue.

(2) To study alterations in receptor number or function in pathological conditions which are characterized by altered receptor sensitivity and in psychiatric diseases.

(3) To provide an experimental model for the study of drug and/or physiologically induced changes in central receptor sensitivity in man.

(4) To understand the membrane perturbations or alterations connecting receptors occupied by their agonists with the inhibition or activation of adenylate cyclases and subsequent physiological events.

Methods Employed:

To measure α-adrenergic receptors, human platelets are prepared from freshly drawn blood. A portion of the platelets is washed and resuspended for measurement of cyclic AMP production. A second portion is homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α-adrenergic antagonist binding. To measure β-adrenergic receptors a leukocyte preparation is made from human blood using a dextran polymer to induce rapid settling of the erythrocytes. The leukocyte-rich plasma is centrifuged at low speed to yield a leukocyte pellet. This pellet is washed several times in 0.9% saline solution and is used to measure binding of a tritiated β-antagonist and production of cAMP.

Cyclic nucleotides are determined by a competitive protein binding assay for cAMP or by radioimmunoassay for cGMP after incubation of intact leukocytes or platelets with the drugs under study. Adenylate cyclase activity is measured in platelet lysates.

Major Findings:

(1) Cyclic AMP production is decreased in platelets from a group of male schizophrenic patients.

(2) Platelets from female patients with idiopathic hypertension have about 1.5 times as many α-adrenergic receptors as platelets from female normotensive control subjects. Platelets from male hypertensive patients produce about one-half as much cAMP as those from normotensive male control subjects.

(3) A decrease in cyclic AMP production was found in two additional groups of male schizophrenic patients.

(4) A decrease in cyclic AMP production was found in a group of female schizophrenic patients.

(5) The number of α-adrenergic receptors is increased in some male and female schizophrenic patients.

(6) The activity of the enzyme adenylate cyclase is decreased in platelet lysates from schizophrenic patients.

(7) α-Adrenergic receptor function is unchanged in platelets from normal men treated with the prostaglandin synthesis inhibitor indomethacin.

(8) β-Adrenergic receptor is decreased in a group of manic and depressed patients as compared with euthymic (control) subjects.

Significance to Biomedical Research and the Program of the Institute:

It is anticipated that results obtained from these experiments will be directly applicable to an assessment of receptor functioning in various human disease states and in monitoring the physiological effects of various drug treatments. If the peripheral α- and β-adrenergic receptors are related to or can serve as a model for central nervous system adrenergic receptors, valuable information may be obtained for the first time about the functioning of these central receptors in psychiatric diseases.

It is possible that adrenergic receptor function in the central and peripheral nervous systems is similar to that measured in platelets and leukocytes. Schizophrenic male patients and some schizophrenic female patients have decreased cAMP production in their platelets. The decrease in cAMP production is due, at least in part, to the decrease in the activity of their platelet adenylate cyclase activity. If neurons in the central nervous system of schizophrenic patients also have decreased cAMP production, there may be a decrease in phosphorylation of, and activation of, some cell proteins, resulting in the deficits that characterize schizophrenia. If the number of α-adrenergic receptors is higher in the neurons, as well as in the platelets, of some schizophrenic patients, transmission in the central nervous system may be altered: norepinephrine or epinephrine occupancy of increased numbers of pre- and post-synaptic α-adrenergic receptors might inhibit brain inhibitory centers, increasing excitation in parts of the brain under tonic inhibitory control. The excitation might contribute to the symptomatology and course of schizophrenia.

β-Adrenergic receptor function in brain neurons may decrease during both mania and depression if these are both stages in the same dimension of illness. The data may also not reflect β-adrenergic function in the brain, but instead a decrease in lymphocyte β-receptor sensitivity in the presence of higher circulating catecholamine levels due to the stress of illness.

Proposed Course:

Examination of the mechanism of information transfer from α- and β-adrenergic receptors to adenylate cyclase is continuing. Studies of these receptors in patients with hypotension, affective illness, and the α-receptor in hyperactive children are underway as well as further studies of α-receptor function in schizophrenia.

Publications:

Exstine, I., Tallman, J., Smith, C.C., and Goodwin, F.K.: Changes in lymphocyte β-adrenergic receptors in depression and mania. Psychiat. Res. 1: 191-197, 1979.

Gullner, H.G., Lake, C.R., Bartter, F.C., and Kafka, M.S.: Effect of inhibition of prostaglandin synthesis on sympathetic nervous system function in man. J. Clin. Endocrinol. Metab. 49: 552-556, 1979.

Kafka, M.S., Lake, C.R., Gullner, H.G., Tallman, J.F., Bartter, F.C., and Fujita, T.: Adrenergic receptor function is different in male and female patients with essential hypertension. Clinical and Experimental Hypertension, 1: 613-627, 1979.

Kafka, M.S., van Kammen, D.P., and Klainman, J.E.: The alpha-adrenergic receptor in man: normal function and pathology. In Usdin, E. (Ed.): Neuroreceptors--Basic and Clinical Aspects. Somerset, N.J., in press.

Kafka, M.S., van Kammen, D.P., and Kleinman, J.E.: Alpha-adrenergic receptor function in schizophrenia. Psychopharmacol. Bull., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00118-04 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Induction of β-Adrenergic Receptors in HeLa Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John F. Tallman	Research Biologist	BP	NIMH
Other:	Richard C. Henneberry	Senior Scientist	LMB	NINCDS
	Craig C. Smith	Chemist	BP	NIMH
	Pierre Mallorga	Visiting Fellow	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, NINCDS
Laboratory of Clinical Science, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into project Z01 MH 00160-01 BP.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00119-04 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Putative Neurotransmitters and Modulators in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John F. Tallman	Biologist	BP NIMH
Other:	Candace B. Pert	Pharmacologist	BP NIMH
	Pauline Lerner	Chemist	BP NIMH
	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH
	Barbara Ewels	Chemist	BP NIMH
	Dieter Naber	Visiting Scientist	BP NIMH
	David Pickar	Staff Psychiatrist	BP NIMH

COOPERATING UNITS (if any)

Unit on Drug Abuse, BP, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.1	PROFESSIONAL: 1.6	OTHER: 2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methods were developed for the determination of cAMP, cGMP, γ-aminobutyric acid (GABA), substance P, endogenous opiate ligands, MHPG, dopamine-β-hydroxylase and cortisol. Using these methods, it is possible to measure these compounds in a variety of tissues and under many physiological conditions. In particular, all of the above compounds may be measured in cerebrospinal fluid and plasma from human subjects. With the available cerebrospinal fluid from patients under study in the Sections of Psychobiology and Neuropsychopharmacology, no differences between patients with schizophrenia and affective disorders have yet been demonstrated for cAMP, cGMP and GABA. Extensive investigation of endogenous opiates and related peptides is underway using both a radioimmunoassay for β-endorphin and opiate receptor assay for other endogenous opiates.

Using radioimmuno, radioreceptor, or radioenzymatic methods it was possible to develop assays for cAMP, cGMP, GABA, substance P and endogenous opiate ligands. cAMP was measured by a protein binding method which utilized protein kinase from bovine adrenal cortex. The cAMP in the sample to be determined competes with radioactive cAMP for a limited number of binding sites on protein kinase. By using varying amounts of unlabeled cAMP, it is possible to construct a standard curve and thus quantitate the amount of cAMP in the sample. Similarly, GABA and endogenous opiate levels were determined by measuring the ability of the sample (or known amount of GABA or endogenous opiate) to displace the radioactive ligand from a brain membrane preparation which binds to GABA or opiates specifically and with a high affinity. Substance P and cGMP were measured using a radioimmunoassay with antibodies either prepared by us or purchased. Antibodies against β-endorphin have been obtained and an assay for β-endorphin has been obtained for examining clinical samples. This assay is good enough to detect the endogenous levels of this compound in body fluids such as plasma or cerebrospinal fluid (CSF). However, reproducibility remains something of a problem. Efforts will continue to improve this antibody and extraction procedure until a successful assay is obtained. At present our assay is capable of determining the levels of β-endorphin after infusions. Other efforts have resulted in the development of a useful radioreceptor assay for β-endorphin which is capable of measuring β-endorphin and other opiates in plasma and CSF.

Dopamine-β-hydroxylase (DBH) and norepinephrine are released from storage vesicles when noradrenergic neurons fire. DBH is being measured in CSF as a possible indicator of central noradrenergic activity. The differential effects of drugs on DBH in CSF and in plasma are also being studied.

3-Methoxy-4-hydroxy-phenylethylglycol (MHPG) is a major metabolite in norepinephrine which can be measured accurately in urine samples by a gas chromatographic assay. MHPG has been measured in urine from unmedicated depressed patients and normal volunteers. There was no significant difference between mean urinary MHPG values for the two groups.

Significance to Biomedical Research and the Program of the Institute:

Measurement of transmitters and modulators of brain function in CSF may be important for understanding the metabolism and turnover of these compounds in brain. If differences exist between groups of patients, it may be possible to implicate a transmitter or biochemical event as being important in affective disorders or schizophrenia.

Proposed Course:

Samples are being assayed to measure endogenous opiates in control states and in patients with different diseases. Assays for cyclic nucleotides are being carried out on a routine basis. An assay for CSF cortisol has been developed and is being applied to the study of CSF cortisol in various patient populations.

Publications:

Naber, D., Pickar, D., Dionne, R.A., Bowie, D.L., Ewels, B., Moody, T.W., Soble, M.G. and Pert, C.B.: Assay of endogenous opiate receptor ligands in human CSF and plasma. Substance and Alcohol Action/Misuse 1: 113-118, 1980.

Tallman, J.F.: Metabolism and Possible Physiological Actions of Endogenous Tyramine. In Mosniam, A.D. and Wolf, M. (Eds.): Noncatecholic Phenylethylamines. New York, Marcel Dekker, 1980, pp. 293-305.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00121-03 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Dopamine-β-hydroxylase in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pauline Lerner	Chemist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into Project Z01 MH 00119-04 BP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00122-03 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Urinary 3-Methoxy-4-hydroxy-phenylethylglycol in Psychiatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pauline Lerner	Chemist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into Project Z01 MH 00119-04 BP.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00123-03 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Blockade by Chronic Lithium Treatment of Presynaptic Dopamine Supersensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dorothy W. Gallager	Senior Staff Fellow	BP NIMH
Other:	William E. Bunney, Jr.	Chief	BP NIMH
	Agu Pert	Psychologist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been completed.

PHS-6040
(Rev. 10-76)

Publications:

Gallager, D.W., and Bunney, W.E. Jr.: Effects of Chronic Lithium Administration on the Development of Supersensitivity in CNS Amine Systems: A Microiontophoretic Study. In Usdin, E., Kopin, I. and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. Oxford, Pergamon Press, 1979, pp. 669-671.

Gallager, D.W., and Bunney, W.E. Jr.: Failure of chronic lithium therapy to block tricyclic antidepressant-induced postsynaptic 5-HT supersensitivity. Arch. Pharmacol., 307: 129-133, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00124-03 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Lithium in the Treatment of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Agu Pert	Psychologist	BP NIMH
Other:	Candace B. Pert	Pharmacologist	BP NIMH
	Jack E. Rosenblatt	Staff Psychiatrist	SMRC NIMH
	William E. Bunney, Jr.	Chief	BP NIMH
	Ellen Silbergeld	Chief, Section on Neurotoxicology	OD NINCDS
	Robert Hruska	Staff Fellow	OD NINCDS

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology, NIMH; Office of the Director, NINCDS

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chronic administration of lithium chloride appears to prevent the development of supersensitivity in the dopaminergic and α- and β-adrenergic central nervous system pathways. While acute administration of lithium has not been found to influence dopamine receptors, chronic administration was found to decrease binding of [^{3}H]spiroperidol by almost 50% in the caudate nucleus if the animals were sacrificed immediately after the termination of treatment. Chronic lithium treatment was also found to decrease the binding of [^{3}H]QNB in the striatum but not in other brain regions indicating an influence on the muscarinic cholinergic receptors at least in some regions of the brain.

PHS-6040
(Rev. 10-76)

It has recently been suggested that oscillations in catecholamine receptor sensitivity may underlie the etiology of affective disorders, especially manic-depressive illness. We have previously found that chronic lithium treatment appeared to prevent the development of dopamine receptor supersensitivity. These findings were taken to suggest that lithium may produce its therapeutic effects by dampening the oscillations in dopamine receptor sensitivity. Subsequent to these initial findings, we also demonstrated similar effects for the α- and β-adrenergic receptor systems. This series of studies was designed to further extend the analysis of the effects of lithium on neurotransmitter receptor sensitivity.

Effects of Chronic Lithium on Dopamine Receptors in the Caudate

In our previous studies we failed to find any effects of lithium on binding of [^{3}H]spiroperidol when it was added to rat brain homogenates. We had also failed to detect any effects of chronic lithium on the binding of [^{3}H]spiroperidol one week following the termination of chronic lithium treatment. In this series of studies we examined the effects of chronic lithium on the binding of [^{3}H]spiroperidol in rat caudate. Rats were fed a diet which was adulterated lithium for three weeks. One group received a high lithium diet while the other group received a medium lithium diet. At the end of three weeks the rats were sacrificed and the caudates dissected out. [^{3}H]Spiroperidol binding was performed as described previously.

Effect of Chronic Lithium on the Muscarinic Cholinergic Receptor

We have previously found an interaction between chronic lithium and cholinergic compounds in the rat. These data suggested that lithium exerted an effect on the cholinergic receptor. In this series of studies we evaluated the effects of chronic lithium on the binding of [^{3}H]QNB (a ligand for the muscarinic cholinergic receptor) in three regions of rat brain. Rats were treated for three weeks with a lithium diet designed to induce lithium plasma levels of ~0.7-0.9 mEq/liter. After three weeks the rats were sacrificed and the brains removed and frozen. The septum, hippocampus, cortex and striatum were dissected out and assayed for [^{3}H]QNB binding.

Major Findings

Although the binding of [^{3}H]spiroperidol was not affected by an acute treatment with lithium and there were not any residual effects of chronic lithium treatment on [^{3}H]spiroperidol binding, when [^{3}H]spiroperidol binding was assessed immediately following lithium treatment there was a significant (50%) decrease in dopamine receptors in the caudate nucleus. This action of lithium on dopamine receptors may account for the anti-manic actions of lithium and may also account for the ability of lithium to antagonize the supersensitivity induced by chronic haloperidol.

In our initial studies it appeared that chronic lithium had induced a significant decrease in [^{3}H]QNB binding in various regions of the brain. However, when lithium was added to the brain homogenate it was also found to inhibit binding to a significant degree. When the rat brain membranes were washed repeatedly to eliminate residual lithium, a decrease in binding was only seen in the striatum. Experiments are in progress to ascertain whether this decrease is truly due to the chronic actions of lithium or whether it is due to acute effects.

Significance to Biomedical Research and the Institute Program

Lithium is one of the most widely used and efficacious compounds in the treatment of mania and manic-depressive illness. Understanding its mechanism of action will aid in defining the factors underlying the expression of mania and manic-depressive illness.

Proposed Course

1. Further attempts will be made to analyze the effects of chronic lithium on other neurotransmitter systems. Special emphasis will be devoted to the enkephalinergic and GABA circuits.

2. A continued effort will be made to assess the effects of lithium on the development of sub- and supersensitivity in the GABAergic, dopaminergic and enkephalinergic systems.

3. An effort will be made to evaluate the effects of lithium on the several populations of dopamine receptors.

Publications

Bunney, W.E., Jr., Pert, A., Rosenblatt, J.E., and Pert, C.B.: Some Biological Considerations of the Mode of Action of Lithium in the Treatment of Affective Illness. In Kline, N.S., Gershon, S. and Schou, M. (Ed.): Proceedings of the International Lithium Conference: Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, in press.

McCulloch, J., Savaki, H.E., Pert, A., Bunney, W.E. Jr., and Sokoloff, L.: Metabolic mapping using 2-deoxyglucose of the effects of the chronic administration of haloperidol and lithium. J. Pharmacol. Exp. Ther., in press.

Pert, C.B., Pert, A., Rosenblatt, J.E., Tallman, J.F., and Bunney, W.E. Jr.: Catecholamine Receptor Stabilization: A Possible Mode of Lithium's Anti-Manic Action. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. London, Pergamon Press, 1979, pp. 583-585.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00143-05 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Physiological Function of Opiate Receptors and Their Endogenous Ligands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Donald L. Bowie	Chemist	BP NIMH
	Barbara Ewells	Chemist	BP NIMH
	Agu Pert	Psychologist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00147-05 BP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Behavioral and Physiological Effects of Morphine-like Brain Peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Agu Pert	Psychologist	BP NIMH
Other:	Michael E. Lewis	Psychologist	BP NIMH
	Candace B. Pert	Pharmacologist	BP NIMH
	Mortimor Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
	Lorenz Ng	Guest Worker	BP NIMH
	Evgeni Bragin	Guest Worker	BP NIMH
	Raymond A. Dionne	Staff Fellow	NA NIDR

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, NIMH; Neurobiology and Anesthesiology Branch, NIDR

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Auricular electroacupuncture in rats is found to increase levels of β-endorphin-like radioreceptor activity in rat cerebrospinal fluid concomitant with increases in pain thresholds. Acupuncture also induced a significant decrease in endorphins in the basomedial hypothalamus, periaqueductal gray matter and medial thalamus. These findings indicate that acupuncture is capable of activating the endorphin systems in several brain regions concerned with pain processing. Foot-shock stress also had a similar effect while cervical probing in female rats decreased endorphins only in the periaqueductal gray matter and the septum. Type I opiate receptors were found to increase along a hierarchical gradient in both the visual and auditory processing systems in the monkey cortex. Type II opiate receptors were distributed evenly among all cortical regions. Type I opiate receptors appear to mediate the depressant actions of opiates in brain while Type II opiate receptors mediate the excitatory and euphorogenic actions.

PHS-6040
(Rev. 10-76)

Objectives

The objectives of this project are to define the mechanisms and sites of action of opiates and opioid peptides and to evaluate the effects of various environmental manipulations on the activity of central nervous system endorphin systems.

Role of Endorphins in Acupuncture Analgesia

Several lines of evidence suggest that acupuncture may induce some of its analgesic actions by activating, at least in part the endogenous pain suppression mechanism encoded by opioid peptides. First, naloxone, a specific opiate antagonist, has been reported to antagonize acupuncture analgesia in both man and rodents. Second, several investigators have found that acupuncture can alleviate certain signs and symptoms of narcotic withdrawal in human addicts as well as rats. We have examined the effects of auricular electroacupuncture in the rat on levels of endorphins in cerebrospinal fluid, brain and plasma. For auricular acupuncture, small alligator clips with 30-gauge needles in the tips were applied to the lower part of the concha just posterior to the external auditory meatus. The animals were then stimulated through these clips with a continuous train of low frequency, low intensity biphasic pulses for 20 minutes. Following stimulation, nociceptive thresholds were tested using the hot-plate technique. Immediately after testing the rats were either decapitated or anesthetized for sampling of cerebrospinal fluid from the cisterna magnum. For brain level determinations the brains were rapidly removed and frozen. The basomedial hypothalamus, medial thalamus, periaqueductal gray matter and the septum plus the bed nuclei of the stria terminalis were punched out from frozen coronal slices. These regions were chosen because they have high concentrations of β-endorphin, enkephalins and opiate receptors and/or because they are associated with pain processing and opiate analgesia.

Effects of Stress and Visceral Stimulation on Brain Endorphins

Stress has also been shown to inhibit nociceptive reactions in rats. Since stress has been shown to increase the levels of both ACTH and β-endorphin in rat plasma, it has been hypothesized that stress-induced analgesia may also be mediated in part by endorphins. We have evaluated changes in levels of endorphins in rat plasma, cerebrospinal fluid and brain following stress. In addition to stress, cervical stimulation of female rats has also been found to induce profound analgesia. We have also examined the effects of this manipulation on brain levels of endorphins.

Regional Distributions of Type I and Type II Opiate Receptors in Brain

There are at least two different subclasses of opiate receptors through which opiates and opioid peptides exert their pharmacological and physiological effects. One class of receptors appears to be coupled to adenylate cyclase (Type I) and the other appears to be uncoupled (Type II). There is a distinct regional distribution for each receptor subclass in brain. Interestingly, regions of the brain that appear to be involved in mediating the analgesic effects of opiates (e.g., periaqueductal gray matter, nucleus gigantocellularis and the spinal cord) have a greater concentration of Type I than Type II receptors. This suggests that the analgesic effects of opiates may be mediated through adenylate cyclase. Type II opiate receptors, on the other hand, appear to be associated predominantly with limbic forebrain structures such as the nucleus accumbens, amygdala and hypothalamus as well as the frontal cortex. Opiate effects mediated by these regions probably include euphoria and hypermotility. Although opiate receptor density is reportedly low in rat cerebral cortex, some regions of monkey cortex have been found to contain a relatively high concentration of opiate receptors. Because of the large literature emphasizing the functional and anatomical heterogeneity of monkey cerebral cortex, a detailed study of the cortical distribution of two types of opiate receptors was carried out. Type I and Type II opiate receptors were labeled under appropriate assay conditions by [^{3}H]naloxone and [^{3}H]D-Ala^2-,D-Leu^5-enkephalin, respectively.

Differential Behavioral Effects of Type I and Type II Opiate Receptor Agonists

Two regions of the rat brain have clearly different proportions of Type I and Type II opiate receptors. The nucleus accumbens appears to have a higher concentration of Type II receptors, whereas the periaqueductal gray matter seems to have a higher concentration of Type I receptors. The caudate nucleus has high concentrations of both Type I and Type II receptors. The aim of this series of studies was to differentiate the behavioral actions of morphine (a prototype Type I receptor agonist) and D-Ala^2,D-Leu^5-enkephalin (a prototype Type II receptor agonist) in these structures.

Major Findings

Electroacupuncture in the rat through electrodes in the ear significantly increased the latency of the hot-plate reactions in all studies. Auricular electroacupuncture sufficient to increase nociceptive thresholds also induced a 60% increase in β-endorphin-like radioreceptor activity in cerebrospinal fluid. Gel filtration chromatography revealed that rat cerebrospinal fluid contained only endogenous opiate ligand material with enkephalin-like molecular weight. We have also failed to detect β-endorphin-like immunoreactivity in rat cerebrospinal fluid utilizing an antibody which recognizes the N-terminal of β-endorphin. By contrast, however, human cerebrospinal fluid has been found by us to contain endogenous opiate ligands with both enkephalin and β-endorphin-like molecular weights. These differences between human and rat cerebrospinal fluid might reflect the relative enrichment of β-endorphinergic neurocircuitry versus enkephalinergic neurocircuitry in human versus rat brain or, alternatively, might merely be due to the fact that cerebrospinal fluid was drawn from different loci--from lumbar region in human and cisternum magnum in rat. Gel filtration chromatography also revealed that endogenous opioids with molecular weights similar to enkephalin are not only increased by acupuncture treatment, but also shift slightly toward higher molecular weight fragments. We speculate that this shift toward higher molecular weight enkephalin-like fragments could be the result of incomplete processing of a longer peptide precursor produced as enkephalinergic neurons are driven to the limits of their production capacity. Concomitant with an increase in cerebrospinal fluid levels of endorphins, there was a decrease in endorphin radioreceptor activity in the medial thalamus, basomedial hypothalamus and the periaqueductal gray matter. The radioimmunoassay revealed a similar decrease in the medial thalamus and the hypothalamus, but failed to detect changes in the periaqueductal gray. We interpret these findings together with the increases in endorphin-like radioreceptor activity in the cerebrospinal fluid to suggest that acupuncture is capable of activating opiatergic pathways in brain resulting in the release of endorphins in various brain loci. While there was a clear influence of electroacupuncture on brain and cerebrospinal fluid levels of endorphins, plasma levels remained unaltered. This finding does not support the proposition that acupuncture produces part of its analgesic effects by activating the hypophyseal endorphin system.

Foot-shock stress was found to induce a significant elevation in nociceptive thresholds. Hypophysectomy was found to attenuate stress-analgesia while adrenalectomy had no effect. ACTH extract did not restore stress analgesia in hypophysectomized rats or enhance it in sham controls. Neither ACTH extract nor $ACTH_{4-10}$ had any analgesic effects following systemic injections. Since ACTH did not restore stress analgesia in hypothysectomized rats or have any analgesic effects alone, it does not appear that this hormone is critically involved in this phenomenon. The glucocortical response to stress, lacking in hypothysectomized rats, also does not appear to be necessary for the expression of stress analgesia since adrenalectomized rats showed a normal analgesic response to stress. We have also found a 50% increase in plasma levels of endorphins using the radioreceptor assay. We have also seen immediate increases (50-100%) in human plasma levels of endorphins following submersion of the hand in ice water (pain stimulus) for one minute. Such increases, however, are several magnitudes too low to account for an analgesic effect. While painful stimuli do appear to increase circulatory levels of endorphins, these increases do not seem to be involved in mediating nociceptive reactions. Stress, however, also altered levels of endorphins in the brain and cerebrospinal fluid. Using the radioreceptor assay, we found decreases of endorphins in the hypothalamus, medial thalamus and the periaqueductal gray matter and no change in the septum and bed nuclei of the stria terminalis. Concomitant with decreases of endorphins in these specific brain regions, we also found significant increases in cerebrospinal fluid levels following stress. In order to ascertain whether β-endorphin or enkephalins were primarily involved in stress analgesia, we evaluated stress analgesia following lesions to the arcuate nucleus of the hypothalamus (the origin of β-endorphin neurons). Since arcuate nucleus lesions did not affect analgesia induced by stress, it appears that the enkephalins and not β-endorphin are involved in this phenomenon. Although naloxone, the specific opiate antagonist, was found to produce a small attenuation of stress analgesia, a much more significant reversal was obtained by lesioning the central nervous system catecholamine neurons with intraventricular 6-hydroxydopamine. Specific lesions to the A-1 nucleus in the brainstem produced an equal antagonism indicating that stress-induced analgesia is dependent on the descending noradrenergic inhibitory pathways to the dorsal horn. Lesions to the serotonergic systems did not appear to have a very pronounced effect, indicating the relative importance of the noradrenergic over the serotonergic pathways.

Cervical stimulation of female rats was also found to decrease the endorphin content of the periaqueductal gray matter. In addition, a decrease was also found in the septum and bed nuclei of the stria terminalis. No effects were seen in the medial thalamus and the hypothalamus. Thus, all three analgesic manipulations appear to alter the activity of the endorphinergic neurons in the periaqueductal gray matter while only acupuncture and stress activate the endorphin systems in the hypothalamus and medial thalamus.

Rhesus monkeys were anesthetized with sodium pentothal before the brains were removed and dissected over ice into 39 areas which could be ordered into several functional hierarchies. For example, in the visual system, neuroanatomical, electrophysiological and behavioral evidence indicates that visual information is processed sequentially through a series of cortical loci corresponding to von Bonin and Bailey areas OC (striate cortex), OB (lunate and inferior occipital sulci), OA (prestriate cortex), TEO (posterior inferotemporal cortex), TE (anterior inferotemporal cortex) and ventral TG (temporal pole). The density of stereospecific Type I opiate receptors increased in a gradient along this system from primary sensory cortex (OC) to the temporal pole regions (TG) which relay sensory information to the amygdala. The relative opiate receptor density observed was 1 (OC), 1.3 (OB), 1.7 (OA and TEO), 2.5 (TE) and 4.2 (ventral TG). A similar progression was apparent in the auditory cortical system, with a 3-fold increase in Type I opiate receptor density in the temporal pole (lateral TG) over that in the supratemporal plane (TB and TG). This pattern of distribution of Type I opiate receptors contrasts markedly with the distribution of Type II opiate receptors labeled by [^{3}H]D-Ala^2,D-Leu^5-enkephalin. The enkephalin labeled receptors were relatively evenly distributed throughout the cerebral cortex, showing less than a 2-fold variation among the 39 regions. Therefore, the two ligands label clearly different populations of opiate receptors in the monkey cerebral cortex.

Intraventricular injections of 1, 5 or 25 mg of D-Ala^2,D-Leu-enkephalin produced a dose-dependent increase in locomotor activity which was only partially antagonized by naloxone. Intraventricular injections of morphine produced a dose-dependent depression of locomotor activity which was antagonized by naloxone. Injections of morphine into the periaqueductal gray matter also produced a dose-dependent depression of locomotor activity, while injections of D-Ala^2,D-Leu^5-enkephalin into the same structure produced no effect or a slight hypermotility. Injections of D-Ala^2,D-Leu^5-enkephalin as well as morphine into the nucleus accumbens increased locomotor activity without depression. D-Ala^2,D-Leu^5-enkephalin, however, was considerably more potent. Injections of D-Ala^2,D-Leu^5-enkephalin into the caudate nucleus, which contains both Type I and Type II receptors, produced contralateral rotational behavior which increased in intensity with repeated injections and was relatively resistant to antagonism by naloxone. Morphine had no apparent effect in this structure. The data suggest that Type I and Type II receptors located in various brain regions mediate different behavioral actions of opiates.

Significance to Biomedical Research and the Institute Program

Since opiates are among the most potent psychomimetic and euphorogenic compounds, it is of special significance that there exist endogenous compounds in brain which are apparently mimicked by opiates. There is substantial evidence that these compounds may be novel neurotransmitters or neuroregulators. If this is the case, then it is quite conceivable that such neurohumors may play an important role in affective states. Furthermore, dysfunctions in the neuronal systems encoded by these peptides may underlie some of the etiology of mental disorders and neurological dysfunctions.

Proposed Course

1. Further attempts will be made to define environmental and physiological conditions that can modify enkephalin levels in brain.

2. Research will be initiated to ascertain the functional significance of opiate receptors in the primate cortex.

3. Efforts will be made to differentiate the behavioral actions of Type I and Type II opiate receptor agonists.

Publications

Bunney, W.E. Jr., Pert, C.B., Klee, W., Costa, E., Pert, A. and Davis, G.C.: Basic and clinical studies of endorphin. Ann. Intern. Med., in press.

Pert, A.: Psychopharmacology of Analgesia and Pain. In Ng, L.K.Y. and Bonica, J.J. (Eds.): Discomfort and Humanitarian Care. New York, Elsevier, in press.

Pert, A., DeWald, L., and Gallager, D.W.: Effects of Opiates on Nigrostriatal Dopaminergic Activity: Electrophysiological and Behavioral Analyses. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. London, Pergamon, 1979, pp. 1041-1043.

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Pert, C.B., Taylor, D.P., Pert, A., Herkenham, M.A., and Kent, J.L.: Biochemical Evidence for Type 1 and Type 2 Opiate Receptors. In Gross, E. and Meienhofer, J. (Eds.): Structure and Biological Function. Rockford, Ill., Pierce Chemical, 1979, pp. 845-851.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00155-03 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Brain Neuropeptide Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

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(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00156-02 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neurotransmitter Receptors in the Sympathetic Nervous System

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into project Z01 MH 00159-01 BP.

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(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00157-02 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies in Benzodiazepine Binding Sites in the Central Nervous System

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the mechanism of action of the benzodiazepines show that benzodiazepines alter neuronal inhibition in the central nervous system through a GABA receptor/benzodiazepine/ionophore complex. Studies on the pharmacological implications for this complex interaction including investigations of GABAergic agents and anticonvulsants such as diphenylhydantoin are described. In addition, studies on the membrane environment and the biochemical characterization of the isolated benzodiazepine binding site have been carried out.

Pharmacological Studies

Studies on the interactions of γ-aminobutyric acid (GABA) and benzodiazepines have been extended to include in vivo labeling of the benzodiazepine binding site in brain by injecting $[^3H]$diazepam intravenously. As previously reported for in vitro binding, the in vivo binding is enhanced by pretreatment of animals with the GABAergic agents amino-oxyacetic acid (AOAA) and muscimol, and the anticonvulsant diphenylhydantoin.

Based on the anticonvulsant activity of the benzodiazepines and GABA-like agents and antagonism of the convulsant agents bicuculline and picrotoxin for benzodiazepine binding, the anti-epileptic agent diphenylhydantoin (DPH) (Dilantin®) was investigated for its effects on the GABA-benzodiazepine system. Using extracellular recording and microiontophoretic techniques, DPH was found to increase the efficacy of benzodiazepines when tested on dorsal raphe neurons in brain. This increased biological effect could be correlated with enhanced specific binding of the benzodiazepine $[^3H]$diazepam. Increases in $[^3H]$diazepam binding are dose dependent following anticonvulsant doses of DPH, while not significantly altered by subconvulsant doses of DPH. In developing rats, effects on brain-specific $[^3H]$diazepam binding were found to parallel the time course for the development of anticonvulsant activity. The enhanced binding of DPH is due to the exposure of cryptic benzodiazepine binding sites. On the other hand, exposure of fetal rats in utero by maternal administration of DPH leads to a diminished number of benzodiazepine binding sites at a certain point in postnatal development. This diminution is correlated with increased susceptibility to seizures in the offspring.

Benzodiazepine-Phospholipid Interactions

In an attempt to understand the events following the occupancy of the benzodiazepine receptor, the possibility that phospholipid metabolism might be altered by benzodiazepines was examined. Cultured astrocytoma cells having specific high-affinity binding sites for benzodiazepines as well as β-adrenergic receptors were used in these studies. Incubation of astrocytoma cells with both β-adrenergic agonists and benzodiazepines results in an additive increase in phospholipid methylation. The data indicate that β-adrenergic receptors and benzodiazepine receptors activate separate pools of phospholipid methylating enzymes and control separate domains of the membrane. Since methylation has been shown to increase membrane fluidity in the rat reticulocyte, it is suggested that an affect in membrane fluidity may be important in the functioning of the GABA/benzodiazepine/ionophore complex. We are presently extending this finding to primary cultures from rat brain.

Benzodiazepine Binding Site Solubilization

To characterize the components of the GABA/benzodiazepine/ionophore complex in the membrane, receptor solubilization studies have been undertaken. The high-affinity binding site for benzodiazepines in mammalian brain has been solubilized using a non-ionic detergent. In a single step approximately 60% of the binding sites are solubilized, while maintaining a high affinity for $[^3H]$diazepam (K_D of 11 nM as compared to 4 nM for the membrane-bound binding sites). The order of potency of benzodiazepines for the solubilized and membrane-bound binding is identical. With the soluble receptor, there is no GABA effect on [^{3}H]diazepam binding. Photoaffinity and alkylating agents for the benzodiazepine site have been developed. [^{3}H]Flunitrazepam will radioactively label binding sites following exposure to ultraviolet light, and this technique will allow us to purify the radioactive receptor and also study receptor turnover in the cultured cells.

Significance to Biomedical Research and Institute Program:

These studies have been directed towards a more complete understanding of the pharmacology and biochemistry of the GABA receptor/benzodiazepine/ionophore complex. Based on electrophysiological and receptor binding studies, it is clear that the benzodiazepines alter neuronal inhibition in the central nervous system. The mechanism for this enhanced inhibition is obviously complex, but studies on membrane environment and on the biochemical characterization of the isolated binding site will lead to a more comprehensive understanding of the mode of action of benzodiazepines and, perhaps, brain mechanisms in general. The benzodiazepines are among the most widely prescribed drugs in the world.

Proposed Course:

Continued studies on the function of the benzodiazepine binding site in neuronal inhibition are expected. We also hope to further characterize the isolated binding site and membrane environment of this receptor.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00158-02 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Benzodiazepine Receptors and Their Endogenous Ligand

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	David Rodbard	Senior Investigator	ERR NICHD
	Laura Russell	Student Scientist	BP NIMH

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00159-01 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neurotransmitter Receptors in the Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Neurotransmitter receptors are being studied in the rat superior cervical ganglion and central nervous system. As neural transmission through the ganglion is modulated by catecholamines, variations in neurotransmitter receptors accompany changes in transmission. Brain neurotransmitter receptors undergo circadian rhythms which may be altered by changed physiological function, the seasons of the year, and drugs.

PHS-6040
(Rev. 10-76)

Objectives:

(1) To develop methods to measure the presence of neurotransmitter receptors in the nervous system.

(2) To measure whether alterations in receptor number accompany alterations in function in the central nervous system and the rat superior cervical ganglion.

(3) To measure rhythms in brain receptors and study their alterations with changes in function and with drugs.

RAT SUPERIOR GANGLION

Methods Employed:

The specific binding of tritiated α-adrenergic antagonist to membranes from the rat superior cervical ganglion is used to measure the number of α-adrenergic receptors. In some experiments ganglia are decentralized (pre-ganglionic fibers from the central nervous system severed) a week before the experiment, removed the day of the experiment and compared with intact ganglia freshly removed from the contralateral side of the rat. In other experiments, ganglia are removed and cultured, and compared with ganglia freshly removed from the rat. Electrical measurements were made post-synaptically with a single electrode and pre-synaptically with suction electrodes.

Major Findings:

(1) Membranes from the rat superior cervical ganglion contain α-adrenergic receptors. [^{3}H]Dihydroergocryptine, an α-adrenergic antagonist, binds specifically, saturably and reversibly to the receptor. Specific binding is defined as that part of total binding displaced by a saturating concentration of phentolamine.

(2) α-Adrenergic agonists l-epinephrine and l-norephinephrine inhibit binding potently, whereas the β-adrenergic agonist l-isoproterenol is weak. α-Adrenergic antagonists phentolamine, dihydroergocryptine, and piperoxane are potent inhibitors of binding, and the β-antagonist dl-propranolol, a weak inhibitor. Binding of [^{3}H]dihydroergocryptine, then, is to an α-, not a β-, adrenergic receptor.

(3) Decentralization decreases the number of α-adrenergic receptors by 50% without a change in K_D, suggesting that the number, rather than the affinity of α-adrenergic receptors, is decreased by decentralization.

(4) Agonist occupancy of α-receptors inhibits Ca^{++}-influx into the pre- and post-ganglionic neurons and inhibits neurotransmission through the ganglion.

Significance to Biomedical Research and the Program of the Institute:

Membranes in the rat superior cervical ganglia contain α-adrenergic receptors. When the axons of cholinergic pre-ganglionic fibers to the ganglion degenerate, 50% of the α-receptors are lost, suggesting that one-half of the α-receptors are present on pre-ganglionic endings located presynaptically with respect to the adrenergic neurons of the ganglion. Electrophysiological experiments show that alpha occupancy of both pre- and post-ganglionic α-receptors inhibits the transmission of a single impulse across the ganglion and modulates the transmission of a train of impulses.

Proposed Course:

α-Receptors on ganglia will be measured during alterations in the ganglion function in organ culture. Also, other neurotransmitter receptors in the ganglia may be measured.

RHYTHMS IN BRAIN NEUROTRANSMITTER RECEPTORS

Methods Employed:

The specific binding of tritiated ligands to membranes prepared from rat brains is used to measure rhythmic changes in receptor binding in rats sacrificed at intervals over a 24-hour period, over the course of the year, with and without physiological alterations and drug treatment. The α-adrenergic receptor is measured with the specific binding of [^{3}H]WB4101; the β-adrenergic receptor, with [^{3}H]dihydroalprenolol; the dopamine receptor, with [^{3}H]spiroperidol; the muscarinic acethylcholine receptor, with [^{3}H]QNB; the opiate receptor, with [^{3}H]naloxone; and the benzodiazepine receptor, with [^{3}H]diazepam.

Major Findings:

(1) There is a diurnal rhythm in the number of α- and β-adrenergic receptors and the dopamine receptor.

(2) The rhythms of all three are altered by chronic treatment with the antidepressant drug, imipramine.

Significance to Biomedical Research and the Program of the Institute:

These experiments document the existence of diurnal rhythms in some brain neurotransmitter receptors. The rhythms are in the number of receptors, not the affinity of each receptor for its tritiated ligand. As transmission across CNS synapses is modulated by receptors, the change in the number of a given receptor, both pre- and post-ganglionically, as well as the relationship between that receptor and other receptors, and the relationship of all to the light-dark cycle could have a profound effect on neuronal activity patterns in the brain. In addition, the alteration of receptor rhythms by chronically administered imipramine may help to elucidate the therapeutic role of imipramine in alleviating depression in patients.

Proposed Course:

The rhythms in other receptors will be measured with and without drugs and physiological alterations. The rhythms in α- and β-adrenergic and dopamine receptors will be measured with other drugs and functional alterations. The relationship of the rhythms to the "biological clock" will be examined.

Publications:

Kafka, M.S., and Thoa, N.G.: Alpha-adrenergic receptors in the rat superior cervical ganglion. Biochem. Pharmacol. 28: 2485-2489, 1979.

Kafka, M.S., Horn, J.P., Thoa, N.B., and McAfee, D.A.: α-Adrenergic receptors in the rat superior cervical ganglion. Bulletin of the New York Academy of Sciences, in press.

Naber, D., Wirz-Justice, A., and Kafka, M.S.: Circadian rhythms of catecholamine receptor binding in the rat brain and their modification by chronic imipramine. Arzneim. Forsch., in press.

Wirz-Justice, A., Wehr, T.A., Goodwin, F.K., Kafka, M.S., Naber, D., Marangos, P., and Cameron, I.: Antidepressant drugs slow circadian rhythms in behavior and brain neurotransmitter receptors. Psychopharmacol. Bull., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00160-01 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Control of β-Adrenergic Receptors in Cultured Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Craig C. Smith	Chemist	BP	NIMH
	Pierre Mallorga	Visiting Fellow	BP	NIMH

COOPERATING UNITS (if any)

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Laboratory of Clinical Science, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

β-Adrenergic receptors are present in HeLa cells and the number of these receptors can be increased by culturing the cells in the presence of butyrate. At low concentrations of butyrate, receptor number is increased and at higher concentrations, both number and ability to activate adenylate cyclase are enhanced. Astrocytoma cells also contain β-adrenergic receptors which are permanently coupled to adenylate cyclase. The β-adrenergic receptor in HeLa can also directly enhance phospholipid methylation, and this effect is not mediated by activation of adenylate cyclase. The amount of phosphatidylcholine in the receptor's microenvironment affects the apparent number of β-adrenergic receptors and the ability to activate adenylate cyclase. Prior exposure of cells to l-isoproterenol causes the cells to become refractory to a second stimulation (desensitized). Additionally, the apparent number of β-receptors decreased. Inhibitors of phospholipase A_2 block desensitization and activators can cause desensitization in the absence of agonist.

Cultured HeLa cells and astrocytoma cells contain binding sites which fit the classification as β-adrenergic receptors. A radioactive β-adrenergic antagonist, l-[^{3}H]dihydroalprenolol, binds to the cells in a rapid, reversible and stereospecific manner consistent with its pharmacological properties. The binding of l-[^{3}H]dihydroalprenolol is of high affinity, and occupancy of this high affinity binding site can be correlated with the activation of adenylate cyclase by known β-adrenergic agonists.

It is possible to induce a number of biochemical changes in HeLa cells by including short-chain, saturated fatty acids in the culture medium. The most effective fatty acid is butyrate, and it is capable of inducing G_{M3} ganglioside synthesis, sialyl-transferase, alkaline phosphatase, α-chain of human-chlorionic gonadotropin, and a particular elongated shape. Additionally, the number, but not affinity, of the l-[^{3}H]dihydroalprenolol binding sites is increased after treating the cells for 24 h with either low (0.5 mM) or high (5 mM) sodium butyrate. Along with the increased number of binding sites, there is an increase in the ability of these sites to activate adenylate cyclase in the presence of the higher butyrate concentration.

Thus, the increase in the number of β-adrenergic binding sites can be separated from the increased "coupling" to adenylate cyclase, since increased receptor number is obtained by culturing the cells at a low butyrate concentration (<1 mM) while higher concentrations of butyrate are required to obtain "coupling" of receptor and adenylate cyclase. Thus, an increased number of receptors can be obtained by preincubating at low butyrate concentrations (0.5 mM); shifting the medium to one containing a higher (5 mM) butyrate concentration results in an efficient coupling to adenylate cyclase. Using this paradigm, a number of factors affecting coupling of receptor and adenylate cyclase have been studied.

In contrast, young astrocytoma cells are always fully coupled and not altered by butyrate. Both cell lines have also proven valuable tools in studies on the involvement of phospholipid methylation in β-adrenergic receptor function. Two asymmetrically distributed membrane-bound enzymes are present in many tissues including reticulocytes and HeLa cells which synthesize phosphatidylcholine by successive N-terminal methylations of phosphatidyl ethanolamine. The first enzyme, methyl transferase I, located on the cytoplasmic side of the plasma membrane, requires Mg^{++}, has a high affinity for the methyl donor S-adenosyl-L-methionine, and catalyzes the formation of phosphatidyl-N-monomethylethylethanolamine. Methyltransferase II, located on the external aspect of the plasma membrane, adds two additional methyl groups to phosphatidyl-N-monomethylethylethanolamine to form phosphatidylcholine. It has a lower affinity for S-adenosyl-L-methionine and does not require Mg^{++}. By these sequential reactions, phosphatidyl ethanolamine, methylated to phosphatidylcholine, can be translocated to the external side of the plasma membrane. This phenomenon may account for the asymmetric distribution of phospholipids in many cells.

It has been shown in intact rat reticulocyte ghosts that activation of β-adrenergic receptors causes activation of both methyltransferases leading to the translocation of phospholipids from the cytoplasmc side of the membrane to the outside. In view of the potential importance of this phenomenon to β-adrenergic receptor function, a series of similar experiments were also carried out in HeLa cells and astrocytoma cells.

In both types of cells the incorporation of methyl groups into the phospholipid fraction is increased in intact butyrate-treated cells in the presence of β-adrenergic agonists. The order of potency of these compounds (l-isoproterenol > l-epinephrine > l-norepinephrine > d-isoproterenol) is consistent with the ability of these compounds to activate adenylate cyclase or cAMP production in intact cells. Activation of methyl group incorporation by the β-adrenergic receptor is extremely rapid, and can be correlated with the receptor occupancy; β-adrenergic antagonists such as l-alprenolol and propranolol block methyl incorporation into the phospholipids, while α-adrenergic antagonists like phentolamine are inactive as antagonists. It should be noted that the increase in methylation is due to receptor occupancy rather than cAMP production because cholera toxin under conditions which will maximally activate adenylate cyclase has no effect on phospholipid methylation.

There is an active system for the turnover of the pool of phosphatidylcholine synthesized in response to β-adrenergic receptor activation. One of the major products in the degradative scheme is a lysolecithin, the product of phospholipase A action on lecithin. This phospholipase is a Ca^{++} requiring enzyme present on the cell surface, and apparently it is also activated in the presence of β-adrenergic agonists. It is not known whether cAMP is required for this activation. Additional products such as free choline have also been observed indicating that additional degradative steps may also be important. Thus, the half-life of the newly synthesized phosphatidylcholine is quite short--also consistent with its involvement in β-adrenergic function.

Methylation of phospholipids affects the exposure of the β-adrenergic receptor on the HeLa cell surface and modulates the ability of this receptor to activate adenylate cyclase. A role of methylation in the modulation of receptor sensitivity is suggested by these studies of phospholipid metabolism. Chronic desensitization of astrocytoma cells by agonists is accompanied by increased turnover of methylated phospholipids and increased release of arachidonic acid. Inhibitors of phospholipase A$_2$ such as mepacrine and tetracaine can block desensitization, while compounds which enhance phospholipase A$_2$ can rapidly cause desensitization in the absence of agonists. This suggests that although there is an immediate increased synthesis of phosphorytidylcholine after receptor occupancy by agonists, the long term net result is depletion of a critical pool of phosphatidylcholine in the microenvironment of the β-adrenergic receptor, leading to receptor desensitization. Recovery of sensitivity may depend on resynthesis of phosphatidylcholine or translocation of the receptor to a new lipid environment.

Significance to Biomedical Research and the Program of the Institute:

The advantages of a model system to study receptor dynamics and function are tremendous compared to studies with animals. As cells in culture which are readily manipulated provide a stable source of cells which respond in a consistent fashion, the effects of drugs at known concentrations at the cell surface may be studied. The examination of drug effects in the model system can lead to predictions of in vivo effects of these drugs in patients. It is also possible to study subsensitivity in this model system which may be related to clinical subsensitive states.

Proposed Course:

Research is currently underway into the following questions:

1. What is the mechanism of catechoalmine-induced subsensitivity?

2. Can we develop receptor- or butyrate-resistant mutants?

3. What is the role of membrane fluidity in desensitization?

Publications:

Extein, I.L., Tallman, J.F., Smith, C.C., and Goodwin, F.K.: Changes in lymphocyte β-adrenergic receptors in depression and mania. Psychiat. Res. 1: 191-199, 1979.

Hirata, F., Tallman, J.F., Henneberry, R., Mallorga, P., Strittmatter, W.J., and Axelrod, J.: Regulation of β-Adrenergic Receptors by Phospholipid Methylation. In Pepeu, G., Kuhar, M., and Enna, S. (Eds.): Receptors for Neurotransmitters and Peptide Hormones. New York, Raven Press, 1980, pp. 91-97.

Parent, J.B., Tallman, J.F., Henneberry, R.C., and Fishman, P.H.: Appearance of β-adrenergic receptors and catecholamine responsive adenylate cyclase activity during fusion of avion embryonic muscle cells. J. Biol. Chem. in press, 1980.

Strittmatter, W.J., Hirata, F., Axelrod, J., Mallorga, P., Tallman, J.F., and Henneberry, R.C.: Benzodiazepine and β-adrenergic receptor ligands independently stimulate phospholipid methylation. Nature, 282: 857-859, 1979.

Tallman, J.F., Henneberry, R.C., Hirata, F., and Axelrod, J.: Control of β-Adrenergic Receptors in HeLa Cells. In Usdin, E. (Ed.): Catecholamines: Basic and Clinical Frontiers. Oxford, Pergamon Press, 1979, pp. 489-491.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00166-01 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Peptide Secretory Products of Oat Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Desmond Carney	Clinical Associate	MO NCI
	John D. Minna	Chief	MO NCI

COOPERATING UNITS (if any)

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LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Oat cell carcinoma is considered to be APUD cells which embryologically give rise to cells which secrete peptides and catecholamines in the central nervous system and periphery. Some patients exhibit psychotic symptoms presumably due to secreted peptides. Characterization of secreted peptides from oat cells in vitro and in vivo is aimed at developing therapeutic strategies and understanding hypersecretory peptide psychological states.

PHS-6040
(Rev. 10-76)

Objective:

1. To locate lines of oat cell carcinomas which secrete unusual peptides, e.g., the endogenous ligands for the valium receptor.

2. To establish whether psychotic symptoms can be produced by secreted peptides.

3. To develop therapeutic strategies based upon metabolic differences in these neuronal-like cells.

Methods Employed:

1. Radioreceptor assays for opiate peptides, vasoactive intestinal peptide and bombesin.

2. Measurement of uptake of catecholamines and their precursors by rapid filtration of oat cells in various incubation media.

Major Findings:

Oat cells cloned in vitro appear to be strikingly sensitive to neurotoxic catecholamine analogs like 6-hydroxydopamine.

Significance to Biomedical Research and the Program of the Institute:

The extensive body of knowledge gleaned toward understanding catecholamine neurons by neuroscientists may be applicable to understanding these neuron-like cancer cells.

Proposed Course:

We plan to demonstrate the mechanism of action of oat cell carcinoma death by 6-hydroxydopamine, etc. Presumably it is due to an avid uptake system because of metabolic dependence on catecholamines and their metabolites, a readily available substrate in the lung where oat cells grow.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00167-01 BP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neurochemical Coding of Brain Pathways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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LAB/BRANCH
Biological Psychiatry Branch

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method has been developed for visualizing brain neurotransmitter or drug receptors by autoradiography. The degree of resolution of this method is so good that it is possible to reconstruct the cellular projections from receptor patterns. Putative projections can be proven by radioimmunoassay or radioreceptor assay of proposed terminals after destruction of cell bodies.

Objective:

To map the neuroanatomical distribution of various chemically coded pathways in brain.

Methods Employed:

1. Newly developed *in vitro* autoradiography--Unfixed frozen brain tissue is melted onto slides, incubated in appropriate radioactive ligands to label receptors, washed thoroughly, lyophilized, fixed with paraformaldehyde vapors and dipped in liquid emulsion for traditional autoradiographic workup.

2. Radioimmunoassay and radioreceptor assay for bombesin and vasoactive intestinal peptide.

Major Findings:

1. Opiate receptors are numerous in sensory systems and within primary sensory nuclei (visual, auditory, olfactory, somatic), they are found always in the superficial, laminated structures.

2. A multisynaptic pathway links olfactory structures with limbic circuits in the amygdala and habenula.

3. Type II opiate receptors, by contrast, have a diffuse distribution suggesting they mark cellular components rather than postsynaptic sites.

4. Distribution of opiate receptors in monkey cortex correlates strikingly well with a previously defined functional hierarchy for sensory processing of the visual and auditory systems.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts by this defined neuroanatomical procedure will enable us to perform lesioning and drug mimicry experiments to determine their functional significance. The method can be used on human brain and ultimately should give information about the contribution of selective degeneration of various neurochemically coded tracts to pathology.

Proposed Course:

We plan to examine opiate receptor distribution in a number of different species, including the human brain, in order to discover if there is a systematic and progressive opiatergic innervation with animal brains of increasing complexity. We also plan to study the distribution of other receptors to reconstruct the neuronal circuitry in a similar manner.

Publications

Herkenham, M., and Pert, C.B.: In vitro autoradiography of opiate receptors in rat brain suggests loci of "opiate-ergic" pathway. Proc. Nat. Acad. Sci. USA, in press.

Moody, T.W., Pert, C.B., and Jacobowitz, D.: Bombesin-like Peptides: Regional Distribution in Rat Brain. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function. Rockford, Ill., Pierce Chemical, 1979, pp. 865-872.

Moody, T.M., Taylor, D.P., and Pert, C.B.: Effects of guanine nucleotides on CNS neuropeptide receptors. J. Supramol. Struct., in press.

Moody, T.M., Thoa, N.B., O'Donohue, T.L., and Pert, C.B.: Bombesin-like peptides in rat brain: localization in synaptosomes and release from hypothalamic slices. Life Sci. 26: 1707-1712, 1980.

Pert, A., Moody, T.W., Pert, C.B., DeWald, L.A., and Rivier, J.: Bombesin: receptor distribution in brain and effects on nociception and locomotor activity. Brain Res., in press.

Pert, C.B., and Herkenham, M.: From Receptors to Brain Circuitry. In Cagan, R. (Ed.): Proceedings of the International Symposium on the Biochemistry of Taste and Olfaction. New York, Academic Press, in press.

Pert, C.B., Taylor, D.P., Pert, A., Herkenham, M.A., and Kent, J.L.: Biochemical Evidence for Type 1 and Type 2 Opiate Receptors. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function. Rockford, Ill., Pierce Chemical, 1979, pp. 845-851.

Pert, C.B., Taylor, D.P., Pert, A., Herkenham, M.A., and Kent, J.L.: Biochemical and Autoradiographical Evidence for Type 1 and Type 2 Opiate Receptors. In Costa, E. and Trabucchi, M. (Eds.): Advances in Biochemical Psychopharmacology, Neural Peptides and Neuronal Communication. New York, Raven Press, 1980, pp. 581-589.

Taylor, D.P., Herkenham, M., and Pert, C.B.: Localization of Specific High-Affinity Binding of Vasoactive Intestinal Polypeptide (VIP) in Rat Brain. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function. Rockford, Ill., Pierce Chemical, 1979, pp. 917-920.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00168-01 BP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Physiological Function of Neurotransmitter Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Susan Gentleman	Pharmacologist	BP NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

Biological Psychiatry Branch

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate receptors are also receptors for naturally occurring peptides called endorphins or enkephalins. Different opiate receptor subtypes like those in the pancreas often mediate opposite physiological effects. Differential ionic and GTP sensitivity of receptor subtypes in vitro reflect the extent of receptor coupling with ionophores and other effectors. In vivo assay of neurotransmitter receptors, capable of detecting altered sensitivity states, has been accomplished in male rats and in the future will be applicable to clinical studies in man.

PHS-6040
(Rev. 10-76)

Objectives:

-- To understand the relationship between obesity, insulin secretion and peripheral endorphin levels.

-- To understand receptor super- and subsensitivity.

Methods Employed:

-- Radioreceptor assay of opiate peptides which retain physiological activity in plasma.

-- Radioimmunoassay of insulin.

-- Radioreceptor assay for benzodiazepines (a new *in vivo* method for measuring β-receptors).

Major Findings:

1. Chronic naltrexone (an opiate antagonist) injections slows weight gain in obese mice with no concurrent effect on lean littermates.

2. Chronic naltrexone simultaneously restores grossly elevated insulin levels toward normal and restores elevated pituitary endorphin levels toward normal as well.

3. Insulin secretion from pancreatic islet cells seems to be under endorphinergic modulation; Type I opiate receptors produce increases in insulin secretion when occupied, while Type II opiate receptors decrease insulin secretion when occupied.
4. Benzodiazepine receptors and GABA receptors share a common chloride ionophore, and the efficiency of their mutual super- and subsensitivity coupling is altered in the supersensitive state.

Significance to Biomedical Research and Program of the Institute:

Opiates produce profound mood-altering, sleep-inducing analgetic effects when administered to humans. These effects are due to an artificial mimicking of the natural effects of endogenous morphine-like factors on their specific membrane opiate receptors scattered throughout the brain and periphery. Diabetes is a general category of many subtypes of disease each with their own molecular mechanism of action involving insulin receptors and insulin secretion. Schizophrenia and/or other affective illnesses may share parallel pathologic mechanisms involving opiate receptors.

Proposed Course:

We plan future experiments on the relationship between obesity and opiate peptides, including the assay of opiate ligands in human plasma, rat plasma and rat cerebrospinal fluid during various cycles of eating. The possibility of interconversion of receptor subtypes to mediate altered states of receptor sensitivity will be explored.

Publications:

DiMicco, J.A., Kopin, I.J., and Pert, C.B.: Evaluation of β-Adrenergic Receptor Status by Plasma sampling in Living Rats. In Usdin, E. (Ed.): Proceedings of the Conference on the Psychopharmacology and Biochemistry of Neurotransmitter Receptors. Amsterdam, Elsevier/North Holland, in press.

Naber, D., Pickar, D., Dionne, R.A., Bowie, D.L., Ewels, B., Moody, T.W., Soble, M.G., and Pert, C.B. Assay of endogenous opiate receptor ligands in human CSF and plasma. Substance and Alcohol Action/Misuse 1: 113-118, 1980.

Peaslee, M.H., Moisset, B., Shibuya, H., and Pert, C.B.: Increase in pituitary melanocyte-stimulating hormone activity of genetically obese (ob/ob) mice. Experientia 36: 133, 1980.

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Rodbard, D., Costa, T., and Pert, C.B.: Is the benzodiazepine receptor coupled to a chloride anion channel? Reply. Nature 280: 173-174, 1979.

Shibuya, H., Gale, K., and Pert, C.B.: "Supersensitivity" to GABA's effect on benzodiazepine receptors develops after striatonigral lesions. Eur. J. Pharmacol. 62: 243-244, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00326-07 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Clinical neuropharmacology and psychobiology of mania and depression.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Jonathan L. Costa	Staff Physician	CN NIMH
	Robert M. Cohen	Staff Physician	CN NIMH
	Iain C. Campbell	Visiting Scientist	CN NIMH
	Thomas Insel	Staff Physician	CN NIMH
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	Larry J. Siever	Staff Physician	CN NIMH
COLLAB:	Monte S. Buchsbaum	Section Chief	BP NIMH
	Edward F. Donnelly	Psychologist	SMR NIMH
	Stanley L. Slater	Staff Physician	DIR NICHD
	Herbert Weingartner	Acting Chief	LPP NIMH

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LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.2	PROFESSIONAL: 3.2	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In further evaluation of our studies of MAO inhibitors, the selective MAO-A inhibitor clorgyline was found to have marked anti-anxiety and anti-dysphoria effects and also to be effective in a different spectrum of depressed patients compared to other MAO inhibitors. Anti-anxiety and antidepressant effects of clorgyline correlated most highly with changes in norepinephrine concentrations in cerebrospinal fluid and plasma. In contrast, the significant reductions produced by clorgyline in CSF levels of 5-HIAA, HVA or MHPG did not correlate with therapeutic responses. In other studies, neuroendocrine studies have contributed to evidence of serotonergic system changes during lithium administration and have added new evidence suggesting that neurotransmitter receptor sensitivity changes may be an important effect of antidepressant drug treatment.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with schizoaffective and characterologic disorders, are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients' self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed on this unit.

2. Biological assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of enzymes, levels of biogenic amines and their metabolites, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and of cortical evoked potentials are accomplished in collaborative studies with investigators in the Biological Psychiatry Branch.

Major Findings:

In further evaluation of data from our comparison of the behavioral and biological effects of the MAO-A selective inhibitor clorgyline and the MAO-B selective inhibitor pargyline which was completed last year, we have reached several new conclusions:

a. Selective MAO inhibition by clorgyline led to anti-anxiety and anti-dysphoria effects which were as prominent and in some cases even more marked than the drug's antidepressant effects. These results may help explain the therapeutic usefulness of other MAOI in "atypical" patients with prominent anxiety-related symptoms, and are consistent with recent studies suggesting a large noradrenergic component in anxiety symptoms.

b. Anti-anxiety and antidepressant responses to clorgyline correlated most highly with changes in norepinephrine in cerebrospinal fluid ($p < .01$) and plasma ($p < .05$). In contrast, the significant reductions produced in CSF levels of 5-HIAA, HVA or MHPG did not correlate with therapeutic responses.

c. Patients with endogenous-type depressions also responded to clorgyline, suggesting that this drug may have a different spectrum of action from other MAOI. It would seem that MAO inhibitors should not be considered a single class of drugs, appropriate for only a single patient group or set of symptoms, as some have suggested.

d. Pargyline had more activating, stimulant-type effects without clear antidepressant effects, and was also rated by observers as producing significant increases in hostility and in total BPRS scores--changes not seen with clorgyline.

e. Although MAO-A constitutes less than 20% of the total MAO activity in human brain, its primary responsibility for the degradation of norepinephrine and serotonin would seem to re-emphasize the role of these neurotransmitters in depression and in the mode of action of the MAOI. In contrast, the minimal effects of pargyline argues against an important role for a phenylethylamine deficit in depression or dopamine in antidepressant efficacy.

f. The 2-3 week delay in onset of antidepressant effects and changes in blood pressure, sleep and biochemical events with the MAOI suggests that neuronal adaptive changes (e.g., the adrenergic receptor changes recently observed in our studies with clorgyline in rodents) may be involved.

In other investigations, Dr. Siever has been examining the effects of clonidine, an α-adrenergic agonist, on patients with primary affective disorders and controls. The growth hormone response to clonidine was measured to evaluate post-synaptic α-adrenergic receptor sensitivity. Other studies have suggested a blunted growth hormone response in depressed patients. We have also measured plasma MHPG, a possible indicator of pre-synaptic α-adrenergic responsivity, and demonstrated a fall in MHPG upon infusion of clonidine. Samples for other hormonal measures such as plasma cortisol, LH, and prolactin, as well as peripheral norepinephrine were drawn. Clonidine was found to consistently lower blood pressure, which was carefully monitored in all patients. We are starting to evaluate changes in the clonidine response with antidepressant treatment with particular interest in elucidating the possible development of pre-synaptic subsensitivity. In connection with studies on noradrenergic receptors, we are also evaluating with Dr. Marion Kafka changes in peripheral platelet α-adrenergic receptors with antidepressant treatment and correlating them with changes in peripheral norepinephrine levels and blood pressure.

Other collaborative studies have examined a number of behavioral and biological changes associated with therapeutic responses to lithium, including personality characteristics such as obsessional features in responding depressed patients, as well as neuroendocrine alterations suggestive of an important contribution to lithium's effects via increased brain serotonergic system function.

Studies of monoamine-related enzymes and monoamine metabolites in platelets, plasma and urine from patients with affective disorders are described in another project report. In addition, newer investigations of neuroendocrine and peptide changes accompanying the administration of agents affecting cholinergic function and mood are presented in another separate research project summary from our Branch.

Publications:

Lake, C.R., Coleman, M.D., Ziegler, M.G., and Murphy, D.L.: Fenfluramine and its effects on the sympathetic nervous system in man. J. Current Med. Res. Opinion 6: 63-72, 1979.

Robinson, D.S., Campbell, I.C., Walker, M., Statham, N.J., Lovenberg, W., and Murphy, D.L.: Effects of chronic monoamine oxidase inhibitor treatment on biogenic amine metabolism in rat brain. Neuropharmacology 18: 771-776, 1979.

Donnelly, E.F., Murphy, D.L., Waldman, I.N.: Obsessionalism and response to lithium. Br. Med. J. 1: 1627, 1979.

Nurnberger, J.I., Gershon, E.S., Murphy, D.L., Buchsbaum, M.S., Goodwin, F.K., Post, R.M., Lake, C.R., Guroff, J.J., and McGinniss, M.H.: Biological and clinical predictors of lithium response in depression. In Cooper, T.B., Gershon, S., Kline, N.S., and Shou, M. (eds.): Lithium, Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, 1979, pp. 241-256.

Murphy, D.L., and Costa, J.L.: Some new approaches to lithium's cellular and metabolic actions of potential relevance to its behavioral effects. In Cooper, T.B., Gershon, S., Kline, N.S., and Shou, M. (eds.): Lithium, Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, 1979, pp. 815-833.

Davis, J.M., Colburn, R., Murphy, D.L., and Robinson, D.S.: The effects of lithium on amine transport and monoamine oxidase activity in brain. In Cooper, T.B., Gershon, S., Kline, N.S., and Shou, M. (eds.): Lithium, Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, 1979, pp. 834-840.

Buchsbaum, M.S., Lavine, R.A., Davis, G.C., Goodwin, F.K., Murphy, D.L., and Post, R.M.: Effects of lithium on somatosensory evoked potentials and prediction of clinical response in patients with affective illness. In Cooper, T.B., Gershon, S., Kline, N.S., and Shou, M. (eds.): Lithium, Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, 1979, pp. 685-702.

Major, L.F., Lake, R.C., Lipper, S., Lerner, P., and Murphy, D.L.: The central noradrenergic system and affective response to MAO inhibitors. Prog. Neuropsychopharmacol. 3: 5-6, 1979.

Karoum, F., Potkin, S.G., Murphy, D.L., and Wyatt, R.J.: Quantitation and metabolism of phenylethylamines and tyramine's three isomers in humans: In Mosnaim, A.D. and Wolf, M.E. (eds.): Noncatecholic Phenylethylamines, Part 2, Chapter 7. New York, Marcel Dekker, 1980, pp. 177-191.

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Donnelly, E.F., Murphy, D.L., and Waldman, I.N.: Denial and somatization as characteristics of bipolar depressed groups. J. Clin. Psychol. 36: 159-162, 1980.

Murray, R.M., and Murphy, D.L.: Can drug response contribute to psychiatric diagnosis and classification? Psychol. Med. (in press).

Cohen, R.M., Campbell, I.C., Cohen, M.R., Torda, T., Pickar, D., Siever, L.J., and Murphy, D.L.: Presynaptic noradrenergic regulation during depression and antidepressant drug treatment. Psychiatry Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00327-08 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Marital Conflict of Manic-Depressive Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C.F. Hoover, D.S.W. Social Worker CN NIMH

COOPERATING UNITS (if any)

VA Hospital, Philadelphia, PA (Dr. R. G. Fitzgerald)

LAB/BRANCH
Clinical Neuropharmacology Branch

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TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of conflict and dominance in marriage compares patients with primary affective disorders, their spouses, and individuals from the community.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study perceptions of dominance in the marriages of patients with affective disorders.

Methods Employed:

The Conflict in Marriage Scale (CIMS), an agree-disagree card sort, was administered to 42 patients and their spouses, as well as to 30 comparable "well" marital pairs from the community. A discriminant function analysis was performed to locate significant differences between patients, spouses, and community individuals. Special emphasis in this phase of the research was on those CIMS items relating to marital dominance as demonstrated in disagreement resolution and decision making. Varying perceptions of husbands and wives were of particular interest.

Marriages of patients with affective disorder, and partnerships of community individuals were compared in terms of mean husband-wife correlations in level of acknowledged conflict. Varying perceptions by husbands and wives who were parents of schizophrenic or maladjusted offspring, as revealed in previous studies by the same investigator, were also reviewed for purposes of comparison.

Major Findings:

Spouses of patients with affective disorders were significantly more often reported to make final decisions on family matters (64%) than patients were (17%). Fifty percent of community women, compared to 3% of community men, reported the partner made final decisions. Yet 78% of women patients and 47% of men patients, but only 21% of women spouses, and 13% of men spouses agreed that the partner made final decisions. Husband-wife correlations of conflict levels reported were lower in the marriages of affectively ill persons than in any other group studied.

These and other findings in relations to varying husband-wife perceptions on dominance and conflict in the marriages of affectively ill persons raise questions concerning possible assortative mating on a basis of complementary personality or dependence needs, as well as speculations concerning the cumulative effect of recurrent affective illness in establishing a characteristic dominance relation between spouses.

Publication:

Hoover, C.F., and Fitzgerald, R.G.: Marital conflict of manic-depressive patients. Arch Gen Psychiatry (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00328-05 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Monoamine Oxidase Activity and Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis L. Murphy	Chief	CN NIMH
OTHER:	Jonathan L. Costa	Staff Physician	CN NIMH
	Iain C. Campbell	Visiting Scientist	CN NIMH
	Larry J. Siever	Staff Physician	CN NIMH
	Ned H. Kalin	Staff Physician	CN NIMH
COLLAB:	Monte S. Buchsbaum	Section Chief	BP NIMH
	Richard J. Wyatt	Director	DSMR NIMH
	Laurence M. Corash	Assistant Chief	CP NIH
	Toomas Eisler	Visiting Scientist	ET NINCDS
	Robert P. Klein	Research Psychologist	CFR NICHD
	Richard Haier	Staff Fellow	LPP NIMH

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Department of Psychiatry, Yale University (Dr. Donald E. Redmond, Jr.)

LAB/BRANCH
Clinical Neuropharmacology Branch

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TOTAL MANYEARS: 3.7	PROFESSIONAL: 2.4	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Previous indications that platelet monoamine oxidase (MAO) activity differences may reflect vulnerability to psychopathology have been supported by further studies in normal populations. A follow-up investigation of individuals identified on the basis of differences in MAO activity as well as cortical evoked response differences revealed a higher incidence of new affective disorders in these same subgroups. Platelet MAO activity differences were also found to be related to other types of vulnerability, including the risks of developing behavioral side effects from disulfiram and MAO-inhibiting antidepressants. In laboratory studies, large primate-rodent species differences in the proportion of the MAO subtypes MAO-A and MAO-B were demonstrated. Brain tissue from three primate species (human, rhesus and vervet) all possessed substantially higher proportions of MAO-B than MAO-A activity across different brain regions, while rats, mice and hamsters all had higher MAO-A activity. These differences raise questions about the applicability of rodent models in studies of some aspects of brain amine metabolism and amine-affective drugs.

Project Description:

Objectives: This project is investigating the apparent relationships between monoamine oxidase (MAO) activity and behavior. Because individual differences in MAO activity are most readily studied in man using blood platelets as sources of the enzyme, the project has also had as an objective the study of factors which affect platelet MAO activity and which may be relevant to the interpretations of differences in MAO activity found in psychiatric patient subgroups. The study of the mechanisms of action of drugs which inhibit MAO activity, and the consequences of MAO inhibition on amine neurotransmitter metabolism and other aspects of brain function are additional objectives.

Methods Employed:

Tissues obtained for the study of monoamine oxidase characteristics include human and other mammalian platelets, brain, heart, liver and kidney. Dr. Redmond has provided platelets and other tissues from vervet and rhesus monkeys as well as several other non-human primate species. Dr. Wyatt and other investigators at St. Elizabeth's Hospital have collaborated on some studies of chronic schizophrenic patients, and Drs. Coursey, Buchsbaum, Haier and Siever have obtained samples from normals. A large number of studies correlating MAO activity with clinical, biological (e.g., urinary MHPG, CSF metabolities, plasma norepinephrine, DBH, plasma prolactin) and pharmacological responses are being conducted on the 6D Unit and collaboratively with other NIMH-IR investigators. Several different MAO assay procedures have been adapted or developed in this laboratory for the study of enzyme activity in platelets and other tissues. Dr. Campbell has evaluated the effects of chronic administration of selective inhibitors of the MAO A and B forms on neurotransmitter amines, amine metabolism and receptor function.

Major Findings:

Reduced Monoamine Oxidase Activity as a Vulnerability Factor for Psychopathology.

We have continued to explore the question of whether reduced monoamine oxidase activity might comprise a biological vulnerability factor for psychopathology, emphasing studies in normal populations and following the biological high risk strategy outlined previously. In a large majority of past reports, chronic schizophrenic patients and patients with bipolar affective disorders and with alcoholism have been found to manifest reductions in platelet MAO activity. Platelet MAO activity is a generally stable characteristic of the individual, and several twin and sibling studies have shown that genetic differences account for most of the inter-individual differences in activity. The well relatives of bipolar patients and the relatives of alcoholic patients also show equivalent reductions in MAO activity, suggesting that this difference represents a familial factor and is not the result of illness. However, reduced MAO activity in family members of bipolar patients does not appear to occur in parallel with illness in these families according to studies recently conducted by Dr. Elliot Gershon and coworkers.

Although the original findings of reduced platelet MAO activity being associated with different psychiatric diagnosis (chronic schizophrenic, alcoholism and bipolar affective disorder) have generally been substantiated in subsequent investigations, the problems of adequately controlling for possible contributions from non-specific elements associated with chronic psychiatric disorders, including drug treatment remain. Hence, we have been continuing to place more emphasis on quasi-epidemiologic investigations in normals and in studies of the well relatives of psychiatric patients.

In an 18-month follow-up study of individuals originally identified as having extremes of low or high platelet MAO activity and further subdivided according to cortical average evoked potential measurements considerably higher incidences of hypomanic and depressive episodes meeting RDC diagnostic criteria were found in the low MAO/augmenting and high MAO/reducing subjects. These same subgroups also had been found to be associated with affective psychopathology in the original retrospective study.

In other studies, platelet MAO activity differences were found to be associated with an elevated risk for the development of behavioral side effects in alcoholic individuals receiving disulfiram. Also, reduced platelet MAO activity has been found to be associated with elevated plasma prolactin levels and with increased eye blink rates in schizophrenic patients. While it has been hypothesized that such differences might reflect alteration in brain rather than peripheral MAO activity in these individuals, no direct evidence in support of this possibility has been forthcoming. Behavioral correlates of differences in platelet MAO activity in rhesus monkeys emerged in a study conducted several years ago by Dr. Redmond, which was just published in full detail in the last year.

In an attempt to pursue preliminary findings of associations between infant cord blood amine oxidase levels and neonatal behavior, Dr. Larry Siever and Dr. Robert Klein initiated a pilot project to study correlations between cord amine oxidase levels at birth with play and relational behavior at 14 months. Eleven individuals from the original neonatal sample were tested in a play session in which novelty-seeking behavior, activity, and response to separation were evaluated. The sample is too small to permit any definite conclusions, but the study is being extended with a new sample of infants from the Bethesda Naval Hospital.

Publications:

Singer, T.P., Von Korff, R.W., and Murphy, D.L. (eds.): Monoamine Oxidase: Structure, Function and Altered Functions. New York, Academic Press, 1979.

Major, L.F., Murphy, D.L., Gershon, E.S., and Brown, G.L.: The role of plasma amine oxidase, platelet monoamine oxidase, and red-cell catechol-O-methyl transferase in severe behavioral reactions to disulfiram. Am. J. Psychiatry 136: 679-684, 1979.

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Eisler, T., Caine, D.B., Ebert, M.H., Kopin, I.J., Ziegler, M.G., Levine, R., and Murphy, D.L.: Biochemical measurements during (-) deprenyl treatment of parkinsonism. In Singer, T.P., Von Korff, R.W., and Murphy, D.L. (Eds.): Monoamine Oxidase: Structure, Function and Altered Functions. New York, Academic Press, 1979, pp. 497-505.

Freed, W.J., Kleinman, J.E., Karson, C.N., Potkin, S.G., Murphy, D.L., and Wyatt, R.J.: Eye-blink rates and platelet monoamine oxidase activity in chronic schizophrenic patients. Biol. Psychiatry 15: 329-332, 1980.

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Haier, R.J., Buchsbaum, M.S., and Murphy, D.L.: An 18-month followup of students biologically at risk for psychiatric problems. Schizophr. Bull. 6: 334-337, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00329-05 CN

PERIOD COVERED October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Platelets as models for the study of neurotransmitter function.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Dennis L. Murphy	Chief	CN NIMH
COLLAB:	K.L. Kirk	Chemist	LC NIAMDD
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	L. Powers	Member of Technical Staff, Bell Lab., Murray Hill	
	E.D. Eanes	Chief, Sec. on Biological Structure	NIDR NIH
	W.K. Warburton	Physicist, Stanford Synchrotron Radiation Lab., Stanford	
	J.R. Lackowicz	Senior Scientist, Freshwater Biological Inst., Navarre	

COOPERATING UNITS (if any)
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LAB/BRANCH Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The processes of amine uptake, amine storage, and amine release have been examined in human and pig platelets. Techniques employed include measurement of fluxes of radiolabelled tracers, fluorescence lifetimes and fluorescence polarization, electron microscopy, and the use of synchrotron radiation to study extended atomic fine structure. The studies extend our understanding of the mechanisms responsible for amine movement across membranes, and the chemical and physical processes associated with and responsible for vesicular amine storage.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Work on this project continues the evaluation of platelet aminergic functions, with special attention to experimental procedures and techniques which provide insight into the biophysical and biochemical bases for amine uptake, storage, and release.

Studies Implemented: (1) Comparison of amine storage capacity in storage vesicles incorporating magnesium (in pig platelets) and calcium (in human platelets); (2) examination of the binding of serotonin to platelets; (3) evaluation of the role of altered osmotic permeability in the release of amine storage vesicles; (4) continued study of the intracellular compartmentation of amines; (5) examination of the properties of the amine uptake system at plasma and vesicle membranes; (6) investigation of the uptake and storage of fluorescent amine analogs, and of the unique properties of this group of substances when stored in vesicles; (7) in vitro preparation and analysis of models for the core of amine storage vesicles; (8) use of synchrotron radiation to examine the extended atomic fine structure of calcium in systems resembling human-platelet storage vesicles.

Methods Employed:

1. General Preparative Procedures. Platelets are isolated from normal volunteers or by exsanguination of NIH pigs. Storage vesicles are separated by differential sucrose-gradient centrifugation of lysed platelets.

2. Examination of Amine Fluxes in Intact Platelets and Isolated Vesicles. Radiolabelled amines are used to measure parameters associated with amine binding, uptake, storage, and release in normal pig and human platelets, in isolated vesicles, and following various experimental manipulations. Formaldehyde fixative is used to stop uptake, and thrombin to release vesicular contents. Release of vesicles is quantitated by electron microscopy of air-dried whole mounts or by the release of radiolabelled serotonin known to reside exclusively in vesicles.

3. Fluorescence and Absorption Studies of Amine Analogs. Platelets are allowed to accumulate various fluorescent and non-fluorescent amine analogs (i.e., quinacrine). The absorbance, fluorescence polarization, and fluorescence lifetimes are then measured, as well as changes in total fluorescence intensity following various treatments known to alter aminergic function.

4. Preparation and Analysis of Models for Amine Storage Vesicles Utilizing an apparatus designed to form calcium precipitates as synthetic analogs of bone mineral, precipitates are prepared with the composition of the calcium and phosphate core of human-platelet storage vesicles. The composition of these precipitates is examined following treatments analagous to the platelet release reaction and the uptake of amines.

5. Extended Atomic Fine Structure of Calcium in Model Systems for Platelet Storage Vesicles. Various types of calcium-containing precipitates, which may

serve as models for human-platelet storage vesicles, are exposed to X-rays above and below the calcium absorption edge. The absorption spectrum of the calcium is recorded and processed by computer to extract information about atomic arrangement of the calcium ligands.

Major Findings:

1. Binding of Serotonin to Intact Human Platelets. When intact human platelets at 0°C are incubated with labelled serotonin and then washed, about 70% of the label appears to cross the plasma membrane and enter the cell. The remainder may be removed by treatments which are believed to act as inhibitors of serotonin uptake, and thus may represent material bound reversibly to the uptake site.

2. Comparison of Amine Uptake, Storage, and Release in Pig Versus Human Platelets. Amine in storage vesicles of pig platelets is associated with adenine nucleotides and magnesium; human-platelet vesicles contain nucleotides, pyrophosphate, and calcium. Possibly because of this compositional difference, the maximal storage capacity of human vesicles for serotonin, dopamine, or quinacrine is approximately 3 times greater than that of pig vesicles. The amine uptake rate in pig platelets is only about 20% of the same rate in human platelets, and the maximal release of vesicles obtainable with calcium ionophores or thrombin is much less in pig than in human platelets. Unlike human platelets, pig platelets apparently fail to store significant quantities of amine in extra-vesicular compartments.

3. Evaluation of the Possible Role of Osmotic Lysis in Exocytotic Release of Storage Vesicles. Treatment of platelets with hypotonic media causes release of vesicular serotonin, but fails to change the size and calcium/phosphorus content of the vesicle core. It thus seems likely that the existence of an osmotic gradient *per se* does not result in exocytosis of vesicles.

4. Continued Study of Amine Uptake by Human Platelets. The plasma-membrane uptake system for serotonin appears to posses several unusual properties. Counter-transport of serotonin does not appear to occur, and the rate of uptake can increase or decrease within seconds of a change in extracellular serotonin concentration (i.e., no transport hysteresis is observed). Elimination or reversal of the intracellular/extracellular sodium gradient slows but does not stop serotonin uptake, suggesting that co-transport of the two molecules is not the predominant driving force for the transport mechanism.

5. Effects of Quinacrine on Amine Uptake and Storage. Quinacrine sequestered inside storage vesicles displays dramatic alterations in its absorption and fluorescence properties, and is rapidly removed by a non-ionophoric, non-exocytotic mechanism following the addition of the ionophore X537A to intact platelets. Quinacrine analogs display similar behavior provided they possess a flat, non-polar aromatic ring and a mobile, more polar aliphatic side-chain bearing an amine moiety. Except at relatively high concentrations, quinacrine itself has no direct effect on serotonin movement across the plasma or vesicle membranes, although the presence of large amounts of quinacrine already inside

vesicles decreases the amount of serotonin which can enter vesicles de novo.

6. In Vitro Preparation and Evaluation of Synthetic Models for Storage Vesicles. Precipitates with the calcium and phosphorus composition of human-platelet storage vesicles can be formed in vitro under appropriate conditions. Because their composition, physical state (amorphous solid), and dissolution behavior parallels that seen for isolated storage vesicles, these precipitates can serve as useful models for examining the role of the vesicle core versus membrane in the uptake and storage of amines.

7. Synchrotron Studies of Synthetic Models for Storage Vesicles. Data has been obtained on the extended atomic fine structure of the calcium in various synthetic models for storage vesicles. Further processing of this information should provide information on the coordination geometry around the calcium atoms, and on the relationship of these molecular structures to the calcium in storage vesicles in intact platelets and after biochemical isolation.

Significance to Biomedical Research and the Program of the Institute: These studies continue the exploration of the factors governing the uptake, storage, and release of amines in platelets, and have extended their scope to include an amine storage system based on magnesium instead of calcium. They have focussed predominantly on transport mechanisms for movement of serotonin across plasma and vesicular membranes, the utility of certain models to describe exocytotic release of vesicles, evaluation of the participation of the vesicle membrane and core in amine storage, and investigation of the molecular architecture of the vesicle components. A more detailed understanding of the biochemistry and biophysics of aminergic function should lay the groundwork for critical evaluation of aminergic function in other systems.

Publications:

Sweetman, H.E., Costa, J.L., Vecchione, J.J., Valeri, C.R., and Shepro, D.: Dense bodies and total calcium in human platelets following aspirin ingestion for a two-week period. Thrombosis Research, 17:55-61, 1980.

Costa, J.L. and Murphy, D.L.: Unique specializations for the subcellar compartmentation of amines in pig and human platelets. In Rotman, A. (ed.): Platelets: Cellular Response Mechanisms and their Biological Significance. England: John Wiley & Sons, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00330-02 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Use of Electron Energy-Loss Spectroscopy to Study Aminergic Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. P. Wall J. Hainfield	Electron Microscopist, Brookhaven National Labs., Brookhaven, LI, NY	
	R. Feder D. Sayre	Senior Physicist, Physical Mathematician	IBM Thomas Watson Research Labs., Yorktown Hts., NY

COOPERATING UNITS (if any)
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TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electron energy-loss spectroscopy, mass thickness measurements, and X-ray microscopy have been used to investigate the distribution of light elements and amines in human platelets. Model systems containing compounds of interest, as well as element-specific images of platelets and other secretory cells, have been used to develop routines for data processing and analysis by computer.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop and utilize techniques which couple imaging of features at a subcellular level with the determination of elemental and molecular composition in small specimen areas. The techniques are then used to study the structure and dynamics of various platelet compartments which sequester amines.

Studies Implemented: (1) Developmental work in cooperation with the BEIB-sponsored NIH facility for electron energy-loss spectroscopy; (2) development and use of methods for the computer processing and display of element-specific images generated by electron energy-loss spectroscopy or X-ray contact microscopy; (3) determination of the mass thickness for electrons of amine storage vesicles in human platelets; (4) use of X-ray contact microscopy to evaluate the composition of amine storage vesicles.

Methods Employed:

General Preparative Procedures: Air-dried platelet whole mounts are prepared following incubation of cells under appropriate conditions, or cells are examined in the hydrated state after varying incubation procedures. Cells are also fixed, dehydrated, embedded, and sectioned in the conventional manner for electron microscopy. Model systems containing various compounds to be studied are prepared by rapid air drying, by slow drying inside collodion sandwiches, or by chemical vapor deposition under ultra-high vacuum.

Developmental Electron Energy-Loss Spectroscopy Studies. Specimens containing fluorinated compounds, or thin films of known composition, are analyzed by obtaining spectra from localized points on the specimen or by imaging for fluorine atoms. Based on the results obtained, microscope operating conditions are adjusted and improved, and computerized processing routings are developed.

Computer Processing of Elemental Images. Negatives of cells or model systems obtained using electron energy-loss spectroscopy or X-ray contact microscopy are digitized and stored on floppy discs. The digital images are then displayed and manipulated in an interactive fashion to extract the desired information.

Measurement of Platelet Mass Thickness: Platelets are examined in a scanning-transmission electron microscope equipped with a field emission gun and special detectors for measurement of electron scattering. The electron-scattering profile is recorded on magnetic tape, and processed by computer.

X-Ray Contact Microscopy Dried and hydrated platelets are exposed to soft X-rays at varying wavelengths, and the absorption image of the cell is recorded in X-ray resist. The resist material is developed and studied in the electron microscope.

Major Findings:

1. Continued Development of Electron Energy-Loss Facility. Work is proceeding on improving the sensitivity of the spectrometer system, converting the microscope data output to a digital one, with interfacing to a computer and storage on magnetic tape for further processing, and on obtaining element-specific images of preparations and model systems. In the interim, the microscope has been used for its electron microprobe capability.

2. Computer Processing and Subtraction of Images to Produce Elemental Maps. Routines have been developed for the subtraction of electron energy-loss images to produce elemental maps of model systems and secretory cells.

3. Measurement of the Mass Thickness of Amine Storage Vesicles Inside Intact Platelets. With appropriate computer processing of electron-microscopic images, it has been possible to calculate the mass thickness of a series of amine storage vesicles in normal human platelets. It should now be possible to correlate variations in this parameter with changes in the amount of serotonin stored, or the addition of other amines, and to study the relationship between membrane area and rate of amine uptake or total amount of amine bound.

4. Ultrastructural and Elemental Analysis by X-Ray Contact Microscopy. By utilizing selected X-ray wavelengths, contact images of platelets can be made which document previous analytical observations on the high calcium, oxygen, and phosphorus content of amine storage vesicles in human platelets. In addition, filaments apparently rich in phosphorus appear to radiate from a peri-cytoplasmic network into pseudopods during platelet spreading and release. By utilizing very thin resists, element-specific images of the platelet can be produced with a resolution of 5-10 nm and studied by transmission electron microscopy. The electron-image pictures of these resists can be digitized and processed by computer (as described above) to provide more quantitative information on the amounts of various elements present.

Significance to Biomedical Research and the Program of the Institute: In the development of techniques to study the subcellular location of elements and compounds in biological systems, it seems clear that a number of experimental approaches should be investigated. Three of the most promising for the analysis of light elements, electron energy-loss spectroscopy, mass thickness measurements, and X-ray microscopy, have been employed utilizing platelets as test systems. The capacity for storing and analyzing data must also be developed in order to utilize all three techniques to their maximum potential, and significant progress has been made in this area as well. An important concomitant of these collaborative efforts, which stand to benefit a number of investigators in NIMH, on the NIH campus, and elsewhere, is that fundamental questions about platelet aminergic function can be posed and addressed.

Publications:

Murphy, D.L. and Costa, J.L.: Some new approaches to the cellular and metabolic actions of lithium of potential relevance to lithium's behavioral effects. In Cooper, T.B., Gershon, S., Kline, N.S., and Shou, M. (eds.): *Lithium Controversies and Unresolved Issues*: Amsterdam: Excerpta Medica, 1979, pp. 815-833.

Douglas, M.A., Hui, S.W., Costa, J.L., and Bailey, J.J.: Computerized processing and subtraction of energy-filtered electron images as an aid to elemental analysis. "38th Ann. Proc. Electron Microscopy Soc. Amer.," San Francisco, CA, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00331-02 CN

PERIOD COVERED October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Use of Nuclear-Magnetic Resonance to Study Aminergic Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	E. Sokoloski	Technician	LC NHLBI
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COLLAB:	F. M. Poulsen	Postdoctoral Fellow, Dept. of Chemistry, Harvard Univ.	
	C. M. Dobson	Assistant Professor, Dept. of Chemistry, Harvard Univ.	

COOPERATING UNITS (if any)

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LAB/BRANCH Clinical Neuropharmacology Branch

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biochemistry and biophysics of amine storage in pig and human platelets have been studied by nuclear magnetic resonance spectroscopy. Amine storage does not appear to be the result of the formation of a macromolecular complex, but rather involves relatively close packing of amine molecules in interstices provided by other vesicle constituents. Examination of model systems indicates the technique will be useful for continued study of amine storage, as well as of the effects of psychoactive drugs and lithium on this process.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Current studies have employed the technique of nuclear magnetic resonance, in conjunction with morphological and biochemical studies, to provide information about aminergic storage mechanisms and their response to psychoactive agents, various biogenic amines, and lithium.

Studies Implemented: (1) Comparison of the amine storage vesicles of pig and human platelets (internal viscosity, possible macromolecular complex formation, chemical characteristics of stored material); (2) changes in the state of pig and human storage vesicles following the addition of biogenic amines or the amine analog quinacrine; (3) setup and testing of a new nuclear magnetic resonance spectrometer (under the joint guidance of NHLBI, DCRT, and NIMH) in order to continue studies of psychoactive drugs and lithium.

Methods Employed:

1. General Preparative Procedures. Large quantities of platelets are isolated by plateletphoresis from normal volunteers or from patients receiving long-term therapy with lithium or other psychoactive agents. Pig platelets are obtained by total exsanguination and differential centrifugation. Cells are incubated according to various protocols, and either prepared for nuclear magnetic resonance study by extracting cells with perchloric acid or by resuspending the cells at a high density, for biochemical evaluation by labelling with radiolabelled amine, or for morphological examination by preparation of air-dried whole mounts.

2. Biochemical and Morphological Studies. The total amount of radiolabelled amine inside platelets is determined by fixing cells with formaldehyde after varying uptake times and initial amine concentrations. Quinacrine is measured fluorometrically. Vesicular material is determined by measuring release induced by thrombin or the calcium ionophore A23187. Amine storage vesicles are counted by electron microscopy.

3. Nuclear Magnetic Resonance Studies of Platelets and Red Blood Cells. Spectra are obtained of cells after varying prior incubation conditions, and compared with those obtained from cell extracts and with preparations of molecules of interest in appropriate model systems. Appropriate pulse sequences are employed to obtain information about molecular tumbling rates, intraorganelle viscosity, and putative molecular weights of any complexes.

4. SetupWof New Spectrometer Facility. Various model systems and compounds are used to examine and improve spectrometer sensitivity, to determine the chemical shifts of molecules and ions in aqueous solution and in various types of complexes, and to relate motional properties to the observed spectra.

Major Findings:

1. Models for Amine Uptake and Storage Deduced from Studies of Pig and Human Platelets. Possibly because of dramatic compositional differences

(magnesium versus calcium-associated), amine storage vesicles from pig and human platelets differ in the biophysical and biochemical mechanism of amine storage. Amines in human platelets apparently become associated with an amorphous, solid complex of calcium, adenine nucleotides, and pyrophosphate. Magnesium, adenine nucleotides, and amines in vesicles of pig platelets assume a gel-like state at 0°C and a highly mobile form at 37°C. Although stored amines in both cases assume the molecular properties of the compartment into which they partition, they apparently do not form a macromolecular complex per se in either of the two storage systems. In addition, a discrete binding site does not appear to be responsible for amine storage, since a variety of amines and analogs can enter and assume similar motional properties, and since the concomitant storage of quinacrine can either change the state of stored material (in pig vesicles) or produce no change (in human vesicles).

2. Setup and Evaluation of the New Spectrometer. A number of model systems have been used to define and improve the specificity and sensitivity of the new spectrometer, and to determine how it can best be used for the study of amine storage and psychoactive drugs. The new facility will be able to examine metal ions directly, permitting evaluation of their chemical and physical state inside storage vesicles, as well as study of the intracellular disposition of the lithium ion in red blood cells and platelets. In addition, various C^{13}-labelled and fluorinated amines, as well as psychoactive drugs (i.e. fluphenazine and fenfluramine) can be examined in platelets and other amine-storing systems.

Significance to Biomedical Research and the Program of the Institute: Nuclear magnetic resonance studies of pig and human platelets have begun to elucidate the contribution of the vesicle core and membrane to amine uptake and vesicular storage, and have delineated the participation of intra-vesicular complexation and binding in this process. The implementation of the new spectrometer should permit continued exploration of amine uptake, storage, and release mechanisms in platelets, the effects of psychoactive drugs and lithium on these processes, and the relationship of this information to similar phenomena in other aminergic systems.

Publications:

Costa, J.L., Dobson, C.M., Kirk, K.L., Poulsen, F.M., Valeri, C.R., and Vecchione, J.J.: Nuclear magnetic resonance studies of blood platelets. Proceedings of Royal Society - Series B, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00332-02 CN

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Animal Models for the Study of Drug Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ned Kalin	Staff Psychiatrist	CN NIMH
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	Iain C. Campbell	Visiting Scientist	CN NIMH
	Robert M. Cohen	Staff Psychiatrist	CN NIMH
	Leslie F. Major	Staff Psychiatrist	CN NIMH
	Samuel C. Risch	Staff Psychiatrist	CN NIMH
COLLAB:	Pauline Lerner	Staff Scientist	BP NIMH
	Michael H. Ebert	Section Chief	LCS NIMH
	Robert M. Post	Section Chief	BP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neuropharmacology Branch

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TOTAL MANYEARS: 1.6	PROFESSIONAL: 0.6	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations in non-human primates have demonstrated a marked circadian pattern of change in cerebrospinal fluid cortisol concentrations. Peak to trough differences in CSF cortisol are greater than those seen in plasma cortisol concentrations. Other neuroendocrine substances (e.g., prolactin and β-endorphin) as well as amines and amine metabolites are being studied in relation to neurotransmitter-related drug effects. Measurement of these same substances in cat and rodent cerebrospinal fluid and brain supplement the studies done in monkeys and man.

Project Description:

Objectives: Rhesus monkeys, cats and rodents are utilized to study the effects of various drugs on cerebrospinal fluid and brain concentrations of neurotransmitters and their metabolites and of neuroendocrine substances to better understand the results obtained from cerebrospinal fluid studies in humans.

Methods Employed:

1. Biological assessment: Cerebrospinal fluid, plasma and platelets are collected for the measurement of hormones, amines and their metabolites, enzyme activities, other chemical variables, and drug levels. Cerebrospinal fluid (CSF) is collected by means of cisternal taps and in some cases by means of indwelling ventricular or lumbar cannulas for continuous flow into a -70°C fraction collector over a 24-hour period.

2. Behavioral assessment: Detailed rating scales of activity, arousal, posturing, appetite, grooming, and aggressiveness were employed both prior to and during treatment with various neurotransmitter-related drugs.

Major Findings:

Cortisol concentrations in the cerebrospinal fluid (CSF) of the rhesus monkey were found to undergo a marked circadian variation. Hourly samples of CSF from four adult male rhesus monkeys showed a mean peak cortisol concentration of 1.56 ± .29 µg% occurring between 0800-0900 hrs and a mean minimum concentration between 2200-0000 hrs of 0.21 ± .06 µg%. The mean daily CSF cortisol concentration increase was 900%, a greater variation than has been reported for plasma cortisol diurnal changes. Although corticosteroids in plasma are well known to vary diurnally in humans and other species, CSF cortisol changes have been little studied. In the single previous study in the literature regarding diurnal changes in CSF cortisol in humans or non-human primates, a diurnal variation in cortisol concentrations was found in humans, although only one normal subject was studied and relatively few data points were available during the 24-hour period.

In an attempt to bridge the gap between human studies where only bodily fluids (CSF and plasma) are studied, and rodent studies in which only plasma and brain tissue are studied, cat plasma and CSF were examined both acutely and chronically and brain tissue analyzed at the termination of a drug trial with clorgyline and pargyline. Aminergic changes in CSF over the course of the 3-week study were followed and then compared to those found in the brain upon conclusion of the study. CSF DBH activity, which is known from our studies in patients to be reduced after four weeks' treatment with MAO-inhibiting antidepressants, was found to not change significantly until 18-21 days of treatment with the MAO inhibitors, approximately the time at which clinical response to these drugs is usually seen. These findings suggest that the clinical response to MAOI's is related not to simple inhibition of MAO, but possibly to some secondary effect of inhibition on the aminergic systems, such as a receptor change. In addition, consistently higher ratios of norepinephrine to DBH activity in CSF were found in the cat, as in the human. This change, which most likely reflects a reduction in central noradrenergic firing rates, is most likely reflective of a change in presynaptic receptor sensitivity.

Publications:

Kalin, N.H., Cohen, R.M., and Murphy, D.L.: Circadian variation in the CSF cortisol concentration of the rhesus monkey. Life Sci. 26: 1485-1487, 1980.

Lerner, P., Major, L.F., Dendel, P.S., Campbell, I.C., and Murphy, D.L.: Central and peripheral dopamine beta hydroxylase: Responses to long term treatment with monoamine oxidase inhibitors. Neuropharmacology (in press).

Lerner, P., Dendel, P.S., and Major, L.F.: Dopamine-β-hydroxylase in cerebrospinal fluid and plasma: Effects of α-adrenergic agents. Brain Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00333-02 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Clinical Neuropharmacology and Psychobiology of Alcoholism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Elliot Shullman	Neurologist, Washington VA Hospital	
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Psychiatry Service, NNMC

LAB/BRANCH Clinical Neuropharmacology Branch

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TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In alcoholic individuals studied during an early stage of alcohol withdrawal following hospital admission, cerebrospinal fluid norepinephrine concentrations were elevated in comparison to those found in the same individuals in a subsequent post-withdrawal, alcohol-free period. In brain samples obtained at autopsy from alcoholic individuals and matched controls, no differences in aldehyde dehydrogenase or monoamine oxidase A or B activities were found, but a significant reduction in brain β-adrenergic receptor numbers and an elevation in GABA receptor numbers were present in the alcoholics.

Project Description:

This project compared a number of biological and psychological characteristics in alcoholics compared to controls. It further investigated some biological changes associated with the alcohol withdrawal state. The effects of chronic alcoholism have also been investigated using brain, liver, and other tissue samples from autopsy specimens.

Methods Employed:

Platelet, plasma, and CSF samples were obtained from alcoholics at both the NNMC and VA hospitals and from control groups at each hospital. Behavioral ratings using the BPRS, Hamilton and Zung Depression and Anxiety Rating Scales, SCL 90, Buss-Durkee Aggression Scale, and MMPI were collected. Demographic information using the ECDEU scales and the RDC were also collected. Multiple autopsy specimens on alcoholics and controls were collected at the VA hospital along with the clinical and neuropathological findings.

Major Findings:

Cerebrospinal fluid (CSF) amines and their metabolites were examined in alcoholic individuals in the early stages of alcohol withdrawal following hospital admission. A significant increase in CSF norepinephrine concentrations was present during this early alcohol withdrawal period compared to values in the same individuals studied two weeks later when the patient no longer was in the withdrawal state. There were no associated changes in cyclic AMP or GABA concentrations in CSF during early alcohol withdrawal.

In several investigations of biochemical measures in brain samples obtained at autopsy from alcoholics and controls, reductions in β-adrenergic receptor numbers and elevations in GABA receptor numbers were observed. No differences in either monoamine oxidase (MAO) type A activity (measured with serotonin as the substrate) or MAO-B activity (measured with phenylethylamine as the substrate were found. This is in contrast to our earlier observations of significant reductions in platelet MAO activity found in both alcoholics and their non-alcoholic relatives in a previous study. Similarly, brain aldehyde dehydrogenase activity was not different in alcoholics compared to controls.

This project has been completed, and final reports in addition to those listed below are in the last stages of completion by Dr. Major, who is now at the State University of New York at Binghamton.

Publications:

Hamm, J.E., Major, L.F., and Brown, G.L.: The quantitative measurement of depression and anxiety in male alcoholics. Am. J. Psychiatry, 136:308-310, 1979.

Lake, C.R., Ziegler, M.G., Major, F.L., Brown, G.L., and Ebert, M.H.: The effects of disulfiram on peripheral and central norepinephrine metabolism and blood pressure. In Fann, W.E., Haracan, I., Pohorny, A.D., and Williams, R.L. (Eds.): Phenomenology and Treatment of Alcoholism. New York, Spectrum Publications (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00334-02 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of the pressor, metabolic and behavioral responses to intravenous tyramine.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An increase in sensitivity to the pressor effects of tyramine, an indirectly acting sympathomimetic agent, is a well known side effect of MAO inhibitor treatment. Using an intravenous tyramine steady state infusion technique we have studied the effects of the selective inhibition of the A and B forms of MAO on tyramine's pressor effects in patients treated with clorgyline, pargyline and deprenyl. Our data have demonstrated that treatment with clorgyline is associated with the greatest increases in tyramine sensitivity, while treatment with pargyline is associated with a lesser increase in tyramine sensitivity. Deprenyl treatment was associated with a negligible alteration in response to tyramine. This data suggests that the tyramine sensitivity which develops during MAO inhibitor administration is principally due to a reduction in MAO type A activity. A high correlation observed between changes in tyramine sensitivity and reduced plasma 3-methoxy,4-hydroxy phenylglycol concentrations supports this interpretation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The purpose of this project is to evaluate the comparative effects of selective MAO inhibitors on the pressor response to tyramine and to evaluate further the biochemical and physiological effects of tyramine infusions. Since selective MAO inhibitors have proven useful in treating depression and hypertension, as well as adjuncts in the treatment of Parkinson's disease, an understanding of their effects on tyramine sensitivity is of great importance. As an indirectly acting sympathomimetic, tyramine is useful for the clinical evaluation of peripheral noradrenergic function.

Methods Employed:

An intravenous steady state tyramine pressor response test is administered to drug free patients and again during the course of treatment with one of the following selective MAO inhibitors: clorgyline (a selective MAO-A inhibitor), pargyline (a partially selective MAO-B inhibitor) and deprenyl (a more selective MAO-B inhibitor). The purpose is to achieve a 30 mm Hg rise in systolic blood pressure and to maintain this elevation for 5-8 minutes. Careful calculation of dosage and rates of infusion permit safe infusions both on and off the MAO inhibitors. The amount of tyramine per minute which maintains the pressor endpoint is the dose endpoint, and provides an index of tyramine pressor sensitivities for comparisons between subjects and treatment periods. Blood sampling is accomplished prior to and during the infusion. Samples are analyzed by enzyme isotope assay for catecholamines and by gas chromatography--mass spectroscopy techniques for MHPG.

Major Findings:

We have found that clorgyline produces a 28-fold increase in sensitivity to tyramine's pressor effects, while pargyline and deprenyl produce considerably lesser increases. Utilizing plasma MHPG as an index of MAO-A activity and platelet MAO activity as an index of MAO-B activity, our data indicates that the greater the inhibition of the MAO-A enzyme, the greater the increase in sensitivity to tyramine. Differences in tyramine sensitivities produced by pargyline probably are attributable to a lesser degree of specificity (i.e., partial A form inhibition) found with this drug.

The time course of the pharmacologic and biochemical changes brought about by the selective inhibitors were also investigated. Our findings suggest that the development of the greatly increased sensitivity to tyramine does not necessarily correspond in time to the onset of enzyme inhibition (monitored by plasma MHPG levels and inhibition of platelet MAO). Tyramine infusions after an acute MAO-inhibiting does of clorgyline show a lesser change in pressor sensitivity than that observed after four weeks of treatment. This portion of the tyramine infusion studies dealing with the effects of selective MAO inhibitors has been completed, and two reports have been submitted for publication.

In a detailed longitudinal study of a cycling bipolar patient, we observed changes in tyramine sensitivity during the course of the switch from depression to hypomania, raising speculation that such mood changes may involve changes in receptor sensitivity. We have also observed that tyramine induced pressor changes in two bipolar patients were associated with shifts from depression to hypomania which lasted as long as the blood pressure elevation, suggesting a possible vulnerability in central mechanisms to inputs from the peripheral noradrenergic systems. Evaluation of data regarding subjective and objectively-rated behavior changes induced by tyramine induced activation of the peripheral noradrenergic system in depressed patients is nearing completion.

Publications:

Pickar, D., Cohen, R. M., Murphy, D. L., and Fried, D.: Tyramine infusions in bipolar illness: Behavioral effects and longitudinal changes in pressor sensitivity. Am. J. Psychiatry 136: 1460-1463, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00335-02 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Smooth Pursuit Eye Tracking Impairment and Its Relation to Psychopathology and CNS Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Larry J. Siever	Staff Fellow	CN NIMH
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	Monte S. Buchsbaum	Section Chief	BP NIMH
	Theodore P. Zahn	Research Psychologist	LPP NIMH
	Douglas Reingold	Biologist	CB NEI
	Leslie E. Brody	Clinical Associate	CN NINCDS

COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Neuropharmacology Branch

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TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.4	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to characterize psychological and psychobiological attributes that are associated with alterations in smooth pursuit eye movements. The smooth pursuit system regulates the eyes' smoothly following a moving target and can be measured by an electro-oculograph recording of an individual watching a moving pendulum. By using the high-risk strategy of screening a large group of college student normal volunteers, individuals with various alterations in smooth pursuit patterns will be identified. Individuals with two common types of alterations will be compared with those with more accurate tracking using a variety of psychological and biological tests to explore the relationship of these patterns to personality, psychophysiological and biochemical variables.

Objectives: This project is designed to study individual variations in smooth pursuit eye movements and their relationship to cognitive controls and personality tendencies. In order to understand such relationships, we are employing the "biological high risk strategy" i.e., identifying individuals from a population of normal volunteers with extreme values of a biologic variable implicated in the etiology of psychiatric disorders and comparing them psychologically with volunteers with normal values of that variable. This strategy has been employed successfully in studies of monoamine oxidase levels in man. College student normal volunteers will be screened to determine the quality of their smooth pursuit tracking. Subgroups of these students showing definite alterations in their smooth pursuit patterns will be compared to those with extremely accurate tracking on a variety of psychological, biochemical, and psychophysiological tests.

Methods Employed:

Volunteers from a local college, after giving informed consent, participate in a screening session testing their smooth pursuit eye movements. They are instructed to follow a moving pendulum, which makes a target excursion of 20 degrees of visual angle and oscillates at 0.4 HZ. Their eye movements are measured via surface electrodes by an electro-oculograph and both eye position and velocity are recorded on paper.

These records are scored for velocity arrests, i.e., points at which the eye stops moving while the target continues, and the pattern of deviation of the eye movements. For example, there are individuals with a high velocity arrest score and "spikey" pattern of eye movements as well as individuals with difficulty initiating eye tracking, so that saccades are substituted for the smooth pursuit movements, i.e., "saccadic substitution." A group of 20 individuals from each of these two groups will be compared with a matched control group exhibiting exceptionally good tracking patterns using a variety of psychological and biological tests as well as a clinical interview and family history.

Major Findings:

Two hundred and eighty volunteers have been screened and the accuracy of their smooth-pursuit eye movements assessed. Twenty individuals with low accuracy tracking, seventy individuals with high accuracy tracking, and ten with "saccadic substitution," have been identified and have undergone extensive psychologic testing including Wexler Adult Intelligence Scale, Rorschach, Minnesota Multiphasic Inventory, and a variety of cognitive and perceptual tests. This testing is almost completed and results have not yet been analyzed. Eighteen of this group of 50 have come to the NIH Clinical Center for psychophysiologic studies with Dr. Buchsbaum, (Continuous Performance Task), and Dr. Zahn (reaction time), neurological exam and EEG with Dr. Brody, and extensive psychiatric interview including the Schedule for Affective Disorder and Schizophrenia (SADS) and family history with Dr. Siever.

In addition, data was evaluated from two pilot samples. The first consisted of normal volunteers coming to the Clinical Center as part of an examination of the relationship between platelet MAO and behavior by studying individuals in the upper and lower decile of a larger volunteer population. Smooth pursuit eye movements were recorded by Dr. Monte Buchsbaum and scored by Dr. Philip Holzman of Harvard University. Although the level of platelet MAO did not correlate with accuracy of tracking, the low accuracy trackers (n=9) even more socially introverted on the MMPI, had less heterosexual contacts per week, had a poorer self-image, and showed greater boundary diffusion on their Rorschach responses then the high accuracy trackers (n=81). In another sample of volunteers (n=24) returning to the Clinical Center as part of a study of the Continuous Performance Task (CPT) and behavior, eye-tracking was measured by Dr. Siever. The accuracy of tracking in this study correlated with elevations on the paranoia, social introversion, psychopathic deviance, and hypochondriais scales of the MMPI, with diminished evoked responses on EEG associated with attentional deficit, and with items on the Wender-Utah Adult Inventory indicating difficulty with relationships and a tendency to act irrationally. There was no correlation between accuracy of tracking and performance on the CPT. This preliminary data was presented by invitation at a symposium at the APA meeting and is being prepared for publication.

Significance to Biomedical Research and the Program of the Institute: The smooth pursuit system has been a focus of interest in psychiatry since it has been demonstrated that it is significantly impaired in psychotic individuals, particularly schizophrenics and some patients with severe affective disorders. This is hypothesized to represent a deficit in involuntary attention or cognitive centering. "Good" and "poor" tracking patterns have been shown to be under genetic control and thus represents one of the few genetically determined biologic variables consistently associated with schizophrenic and other psychoses. By using the high-risk strategy of screening a college population for alterations in this system, the relationship of this variable to qualities of attention and personality tendencies in a non-psychiatrically defined population might be clarified without the confounding variables of the length of illness, medication, or hospitalization. This may facilitate delineating a dimension of psychological function closely tied to involuntary attention as reflected in eye-tracking performance.

Proposed Course: We will continue to study the 50 selected volunteers until they have all undergone psychophysiological and neurologic testing, a psychiatric interview, and repeat eye-tracking evaluation. In addition, blood will be drawn for monoamine oxidase and dopamine-β-hydroxylase activity measurements.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00336-01 CN

PERIOD COVERED October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Dennis L. Murphy	Chief	CN NIMH
COLLAB:	Ned H. Kalin	Staff Physician	CN NIMH
	S. Craig Risch	Staff Physician	CN NIMH
	Larry J. Siever	Staff Physician	CN NIMH
	Irwin J. Kopin	Chief	LCS NIMH
	J. Christian Gillin	Deputy Chief	SMRC NIMH
	Monte S. Buchsbaum	Section Chief	BP NIMH
	Carol F. Hoover	Social Worker	CN NIMH
	Robert N. Elkins	Guest Worker	BP NIMH
	Judith L. Rapoport	Unit Chief	BP NIMH

COOPERATING UNITS (if any)

Robert D. Coursey, Ph.D., Dept. of Psychology, Univ. of Maryland

LAB/BRANCH Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obsessive-compulsive disorder is an uncommon syndrome about which little is known. The purpose of this study is to collect basic data on the phenomenology, neuropsychology, and treatment of this disorder. Such biologic measures as sleep, monoamine metabolites, averaged evoked potentials, and galvanic skin response are being studied in relation to symptoms and behavior in these patients. Treatment with clorgyline, a selective MAO inhibitor, chlorimipramine, a tricyclic antidepressant and placebo are being compared.

PHS-6040
(Rev. 10-76)

Project Description;

Objectives: The current study addressed four different sorts of questions. The first focuses on the phenomenological question: Is this a heterogeneous syndrome and, if so, how can we identify different subtypes? Related issues are as follows: What is the relationship of objectively measured anxiety to symptoms; e.g., do patients with rituals have more or less anxiety after performing their compulsive behavior? Is there a cyclic rhythm to the symptoms as has been described in the stereotypies of schizophrenics? A second set of questions relates to the neuropsychology of this disorder: Do some of these patients show abnormal findings on neurological exam, cognitive testing, laterality tasks, or CAT scans? How might such patients differ symptomatically from those without neuropathological findings? A third question deals with etiology, namely, are there genetic markers that correlate with the phenotypic expression of the disorder? This is particularly of interest in families where multiple individuals are affected. Finally, there is the neuropharmacologic question: Is clorgyline as effective as chlorimipramine in treating obsessive-compulsive disorder, and, if so, how is its effectiveness correlated to serotonin uptake changes measured directly (in platelets) and indirectly (with cerebrospinal fluid metabolites)?

The purpose of the present investigation, then, is to develop a more comprehensive picture of the phenomenology, the neuropsychology, the genetics, and the neuropharmacology of this syndrome.

Methods Employed:

Obsessive-compulsive subjects are studied in both inpatient and outpatient settings. Patients who apply for the protocol are carefully screened with an extensive psychological and medical battery. Those accepted must have obsessive or compulsive symptoms for at least one year independent of another psychiatric diagnosis. An initial evaluation includes cognitive, projective, psycholinguistic and psychophysiologic (EEG, ESR) testing. Biologic measures which have proved useful in studies of affective or schizophrenic disorder are focused on here: sleep physiology, average evoked response, urinary and cerebrospinal fluid monoamine metabolites, a variety of platelet and RBC enzymes, and the dexamethasone suppression test are all studied. Cerebral CT scans are done to investigate the possibility of gross neuropathology in this disorder. A major aspect of the study is the comparative double blind drug trials. These trials follow a randomized cross-over design with assessment by both self and observer ratings on a weekly basis. Specific biological and psychological measures are repeated during the various stages of the drug trials for correlation with clinical state.

Major Findings and Proposed Course:

This study is still in the beginning stages. Thus far, much of our effort has been devoted to recruitment of patients and to the development of rating scales. Two subjects have completed the full evaluation and are currently involved in the cross-over drug trials. Four others have participated in various parts of the initial evaluation studies, including sleep studies, psycholinguistic evaluation evoked potential measures.

It is too early to draw any conclusions from our pilot studies to date. However, we have been surprised in this early stage by the frequency of soft neurologic signs in this cohort. We hope to focus on this question in a more specific way with a greater number of subjects in the coming year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00337-01 CN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Neuropharmacology of Neuroendocrine Regulatory Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Ned H. Kalin	Staff Physician	CN NIMH
	Samuel C. Risch	Staff Physician	CN NIMH
	Thomas R. Insel	Staff Physician	CN NIMH
COLLAB:	David Pickar	Chief, Unit on Drug Abuse	BP NIMH
	Dieter Naber	Visiting Scientist	BP NIMH
	Gary W. Kraemer	Investigator, Wisconsin Primate Lab., Univ. of Wisconsin	
	William T. McKinney	Professor, Dept. of Psychiatry, Univ. of Wisconsin	

COOPERATING UNITS (if any)
Wisconsin Primate Laboratory, University of Wisconsin

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
ADAMHA, NIH, NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	2.1	0.6

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of radioimmunoassays have been adapted for use in our laboratory, enabling rapid determinations of ACTH, cortisol, prolactin, beta-endorphin, and growth hormone levels in plasma and cerebrospinal fluid of monkeys and man. With these assays, we have initiated a series of studies designed to characterize the diurnal rhythms and regulatory mechanisms of a number of hormones which reflect hypothalamic-pituitary activity in rhesus monkeys and man. A specific circadian cortisol rhythm has been characterized in the CSF of monkeys similar to that previously reported for man. Biologically active opioid peptides in CSF of rhesus monkeys and human plasma have been demonstrated to have a similar diurnal pattern to that of ACTH. A low dose-response relationship has been determined for dexamethasone suppression of plasma cortisol levels in monkeys. At levels of dexamethasone sufficient to suppress plasma cortisol levels, β-endorphin immunoreactivity in plasma remains unchanged, suggesting some previously unappreciated differences in the regulation of the secretion of these two hormones. In addition, we have initiated studies of the neuroendocrine changes accompanying specific pharmacologic challenges, e.g. neuroendocrine changes accompanying development of the physostigmine behavioral syndrome in man.

Project Description:

Objectives: The limbic-hypothalamic-pituitary axis plays an important role in the response to stress in both animals and man. In addition, dysregu lation of this axis has been demonstrated in some depressed patients. This project is investigating the underlying neurotransmitter-related mechanisms o regulation in this hormonal axis. The study of these mechanisms have a poten tially explanatory role in the understanding of animal models of stress vulne ability, including those leading to the production of depression in monkeys. These models, some of which are based on neuropharmacologic challenges which can also be carried out in man, may also provide direct insights into human affective disorders. Rhesus monkeys can be usefully employed to evaluate hormone responses in the CSF of higher primates, in order to better evaluate the significance of changes in plasma responses to brain responses.

Methods Employed:

Neuroendocrine measurements: Monkey cerebrospinal fluid is collected b means of indwelling lumbar cannulae for continuous flow into a refrigerated fraction collector. Monkey plasma is obtained either by use of indwelling venous catheters or by femoral venipuncture following ketamine-induced anesthesia. Human samples are obtained by maintenance of a heparin lock in t subject's forearm, allowing frequent sampling with minimal stress. Cortisol, growth hormone, prolactin, ACTH, and β-endorphin are assayed by competitive binding utilizing measured displacement of labelled competitive binding trace from their specific antibodies. Biologically active opioid peptides are measured by competition with (H^3)-$(D\text{-}Ala)^2$ enkephalin-L-(leu-amide) for bind sites on crude rat brain membranes.

Psychological and Behavioral Assessment: Pretreatment evaluation of patients and volunteers is accomplished through interviews of the individual and family members as well as assessment of past medical records. When appropriate, structured Affective Disorders Interviews (SADS) are used and Research Diagnostic Criteria (RDC) diagnoses made. Direct behavioral observa tions are performed by both nursing staff and physicians by the use of quanti tive rating scales. Subjects themselves fill out appropriate ratings. Thes approaches enable us to attempt to correlate specific neuroendocrine finding with diagnostic categories and/or behavioral changes observed spontaneously o in response to pharmacologic challenges or antidepressant treatments.

Major Findings:

Studies of Hormonal Diurnal Variation: In a series of studies of plasm and cerebrospinal fluid hormonal levels coordinated by Dr. Ned Kalin, cortis was found in rhesus monkey CSF and demonstrated to have a smooth 24 hour sec tory pattern with a peak occuring between 0800 and 0900 hours and a trough between 2200-0000 hours. An average peak to trough change of 900% was observ These changes over the 24 hour period in CSF may be expected to more adequate represent CNS exposure to cortisol levels, as CSF cortisol seems to correspo more closely to the free plasma cortisol fraction. In collaboration with Dr Naber and Pickar of the NIMH Biological Psychiatry Branch we have found a sin diurnal pattern for biologically active opioid peptides in the CSF of monkeys and in human plasma. The highest levels measured in both groups were between 0600 and 1000 with additional smaller peaks occuring every 6 to 8 hours. The findings are important for the interpretation of CSF and plasma level studies of psychiatric populations where changes in circadian pattern might be expected. Although this data provides further support for the close link hypothesized to exist between ACTH secretion and that of β-endorphin, further observations based on physostigmine infusion and dexamethasone suppression have suggested a possible dissociation in the regulation of these two systems.

Dexamethasone Suppression Studies: Dr. Kalin, in collaboration with Drs. Kraemer and McKinney, has characterized a log-dose response relationship between 11:00 p.m. dexamethasone administration and suppression of 8:00 a.m. plasma cortisol levels. The lowest dose, (0.75 μg/kg) yielded no significant suppression and the highest dose (33 μg/kg) produced a maximal suppression of 25% of baseline; the same maximal effect was achieved with a 17 μg/kg dose. Dexamethasone suppressibility varied with age of the animals as well as with particular environmental and genetic factors. As part of the study, the anaesthetic ketamine was demonstrated to have no net effect on plasma cortisol levels over a period of 3 to 30 minutes, and on β-endorphin immunoreactivity levels up to 20 minutes. Using a dose of dexamethasone sufficient to suppress plasma cortisol levels in both monkeys and man, β-endorphin values show no change. This finding of a cortisol β-endorphin dissociation suggests that previous findings of dexamethasone suppression of β-endorphin plasma levels in rats may have resulted from the much larger doses of dexamethasone utilized, or that differences exist in β-endorphin regulation in primates and rodents.

The dose response parameters determined now give us an opportunity to look at the individual responsiveness of the limbic-hypothalamic pituitary system to negative feedback. This information may prove relevant to individual vulnerability to stress or depression, or to such changes that occur in this system in some individuals undergoing affective state change.

The Physostigmine Syndrome: Coordinated by Dr. Samuel Risch, intravenous infusions of physostigmine, (0.02 mg/kg), a mixed nicotine-muscarinic cholinergic agonist or saline were administered to normal volunteers in a randomized double-blind paradigm, with all subjects receiving methscopolamine prior to infusion to ameliorate peripheral, but not central cholinomimetic effects. Frequent plasma samples were obtained as well as concurrent subjective and objective behavioral ratings. As had only been previously reported for patients with affective disorders, a typical "physostigmine syndrome" consisting of decreased speech, slowed thoughts, mild sedation, occasional nausea, and decreased spontaneous activity was observed. Elevations in plasma cortisol, prolactin and β-endorphin levels were found. However, changes in cortisol levels did not correlate with changes in β-endorphin, as one might have expected from the literature. Only β-endorphin changes, of all the hormones measured, were significantly correlated with the mood changes observed. As enhanced cholinergic sensitivity has been reported in affectively ill patients, the infusion of cholinergic agonists accompanied by the study of changes induced within normal humans and monkeys may provide important new insights into the hormonal mechanisms involved in the development of affective changes, including depression.

Publications:

Risch, S.C., Cohen, R.M., Janowsky, D.S., Kalin, N.H., and Murphy, D.L.: Plasma β-endorphin and cortisol elevations accompany the mood and behavioral effects of physostigmine in man. Science (in press).

Kalin, N.H., Risch, S.C., Cohen, R.M., Insel, T., and Murphy, D.L.: Dexamethasone fails to suppress β-endorphin plasma levels in rhesus monkeys and man. Science (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00446-11 CP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Inpatient Clinical Studies of Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frederick Goodwin	Chief	CP/NIMH
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	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinol.	CP/NIMH
	Alfred Lewy	Staff Psychiatrist	CP/NIMH
	William Potter	Asst. to the Branch Chief	CP/NIMH
	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
	Steven Paul	Chief, Unit on Neuropharmacology	CP/NIMH
	Helena Calil	Guest Worker	CP/NIMH
	Robert Post	Chief, Clinical Res. Unit	BPB/NIMH
	Dennis Murphy	Chief	CN/NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch, NIMH; Clinical Neuropharmacology Branch, NIMH

LAB/BRANCH
Clinical Psychobiology Branch

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NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorders, particularly with regard to the complex interrelationships among clinical phenomenology, diagnosis, biological variables, and pharmacologic response. The studies are both cross-sectional and longitudinal; drug trials are double-blind. This past year, a new program has been initiated emphasizing a brief pre-treatment inpatient evaluation of patients with a variety of endogenous and non-endogenous depressive syndromes, followed by outpatient treatment. Meanwhile, longitudinal inpatient studies of endogenous depression, mania, and rapid-cycling bipolar illness continue. Major projects include the study of circadian rhythms, neuroendocrine substances and monoamine neurotransmitters and their metabolites, both during untreated affective episodes and following specific treatment. The primary treatment modalities include 1) environmental manipulations, such as sleep deprivation or sleep shifts, 2) tricyclic antidepressants showing specificity for given neurotransmitter systems, and 3) electroconvulsive therapy (ECT).

Project Description:

This description attempts to provide a comprehensive view of the inpatient clinical studies of manic-depressive illness. In this group of studies the overall objective is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorders, particularly in relation to specificity of diagnosis, existence of clinically or biologically identifiable subgroups, the nature of predisposing factors, and the interrelationship between pharmacological and psychodynamic factors in clinical improvement.

Traditionally, the focus of our specialized resources has been the longitudinal study of episodes of affective illness, before, during and often following a variety of therapeutic interventions. Initially, studies focused on classical, discrete episodes of mania or endogenous depression; more recently, our inpatient resources have been devoted increasingly to individuals with rapid-cycling bipolar illness. During the past year, a new dimension has been added to the inpatient studies, with the introduction of a "brief inpatient evaluation" program for depression, in which outpatients are admitted to the unit for one week of pre-treatment studies and are then discharged to be treated as outpatients (see Project Z01 MH 01852-02 CP). This approach enables us to evaluate a much broader spectrum of depressive disorders, using the methodologies previously applied to the endogenous depressions, without seriously compromising our ongoing longitudinal studies of entire illness episodes.

Central to an effective program of inpatient studies are regular and reliable procedures for gathering data. A variety of variables are monitored continually during a patient's hospital stay. Trained nursing staff members complete twice-daily global ratings, assessing depression, mania, psychosis, anger, and anxiety. Twice a week a modified form of the Brief Psychiatric Rating Scale (BPRS) is scored for each patient. Patients complete self-rating forms twice a day. Specialized scales, such as the Hamilton Depression scale, the Beck depression inventory, or the Symptom Check List (SCL-90), are used for particular studies. Information regarding families is obtained through ongoing contact between the social worker and the patient's relatives.

Behavioral data are continually collected and quantified. Sleep is recorded at half-hour intervals throughout the night. Motor activity is recorded continuously using solid-state wrist activity monitors (See Project Z01 MH 00450-06 CP), allowing the application of sophisticated mathematical techniques such as spectral analysis to define hitherto unrecognizable patterns in the data. Finally, samples of plasma and urine are collected at regular intervals and stored for subsequent analysis, providing a "bank" of biochemical specimens from various mood states and treatment.

In addition, specific cross-sectional studies are scheduled to provide information about the patient's pre-treatment biochemical state and the biochemical changes associated with changes in mood and treatment modality.

Among these cross-sectional studies are: baseline neuroendocrine values, circadian neuroendocrine patterns, and responses to neuroendocrine challenges (dopamine, saline, and TRH infusions) (See Project Z01 MH 00452-05 CP); circadian patterns of activity and temperature (See Project Z01 MH 00450-06 CP); sleep recordings; and cerebrospinal fluid and urinary monoamines and monoamine metabolites (See Project Z01 MH 00447-11 CP).

Since descriptive and diagnostic dimensions are crucial to the meaningful interpretation of psychobiologic data, work on the standarization of these dimensions has continued. Use of the Research Diagnostic Criteria (RDC) (described in detail in the July 1976 - September 1977 report on Project Z01 MH 00446-08 CP) is now routine. Discharge review conferences are used to establish both a final RDC diagnosis and assess the extent to which each patient has "typical endogenous" depressive episodes. In order to facilitate this task a subjective "typicality" scale is assessed and a record of specific endogenous features is made.

After the evaluation phase of the hospitalization, a variety of investigational interventions are employed. Environmental manipulations have played an increasingly prominent role in the overall research program. Examples include:

1. Sleep Shifts have been successfully used in one rapid cycling patient to produce several switches out of depression. Patients with initial insomnia but otherwise normal sleep patterns have been successfully treated by gradually shifting their sleep period later in the day until it coincides with the "normal" period of sleep. Thus, some insomniacs may be treatable with specific non-pharmacologic interventions.

2. Sleep deprivation induces a brief remission in many depressed patients. Correlates of this remission are being explored.

3. Exposure to light during non-daylight hours has been used to study the effects of the light-dark cycle on neuroendocrine functioning and circadian rhythms.

A variety of pharmacologic studies continue on the inpatient unit. All drug trials are conducted under double-blind conditions with alternating active and placebo periods in a non-random design. The use of placebo substitution following active drug increases one's level of confidence in the efficacy of a compound in an individual patient. This strategy is important in the evaluation od drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness.

1. The main antidepressant drug trial under way on the ward involves a crossover between zimelidine and desmethylimipramine, the former having highly selective effects on reuptake in brain serotonin systems, while the latter is highly specific for norepinephrine systems (See Project No. Z01 MH 01850-03 CP).

2. Mania has been treated with propranolol, with limited benefits (See Project Z01 MH 01851-03 CP).

3. Rapid-cycling bipolar illness has proven to be difficult to treat. Current interventions include: discontinuation of tricyclic antidepressants, treatment with lithium, and the use of low doses of monoamine oxidase inhibitors. Recent findings linking rapid cycling with hypothyroidism (See Project Z01 MH 00449-06 CP) suggest a further therapeutic trial of thyroid hormone is warranted.

An extension of previous studies of the behavioral and biochemical effects of electroconvulsive therapy (ECT) is under way, with increased emphasis on neuroendocrine and circadian parameters.

Results of these interventions are described in greater detail in the individual project reports.

Finally, "normal volunteers" live on the inpatient unit for varying periods of time to participate in specific investigational studies. During the past year, they have been involved in studies of the effects of light and of sleep shifts on neuroendocrine functioning and various circadian rhythms (See Project Z01 MH 00450-06 CP) and in studies of the biochemical and behavioral effects of desipramine and of DDAVP (a vasopressin analog).

Publications:

Zis, A.P., and Goodwin, F.K.: Novel antidepressants and the biogenic amine hypothesis of depression: the case for iprindole and mianserin. Arch. Gen. Psychiatry 36: 1097-1107, 1979.

Zis, A.P. and Goodwin, F.K.: Behavioral effects of L-DOPA in man. In Shader, R.I. (Ed.): Psychiatric Complications of Medical Drugs. New York, Raven Press, in press.

Goodwin, F.K.: The crisis in psychiatric research. In Ayd, F.K., Jr. (Ed.): Crisis In American Psychiatry. Baltimore, Maryland, Ayd Medical Communications, Ltd., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00450-06 CP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biological Rhythms in Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Basic research in biological rhythms can be related to affective illness with respect to (1) the inherent cyclicity of the illness (itself a type of biological rhythm) and (2) the involvement of disturbed circadian rhythms (24-hour cycles) in its pathophysiology. This project is designed to explore these connections by interrelating behavioral, physiological and biochemical changes observed (1) through the cycle of the illness, and (2) through the course of the 24-hour day. Results to date indicate (1) in depression the timing of circadian rhythms is abnormally early (phase-advanced) relative to the day-night cycle; (2) normals' sleep, temperature patterns and neuroendocrine function can be made to resemble that of depressives by shifting their sleep period 6 hours later than usual; (3) some depressives' circadian rhythm phase-advance and mood disturbances can be temporarily corrected by shifting their sleep period 6 hours earlier than usual; (4) animal studies in our laboratory indicate that the two major classes of drugs which are used to treat depression may act by slowing the intrinsic rhythm of circadian pacemakers causing a corrective delay in depressives' abnormally advanced circadian rhythms, (5) in mania the 24-hour sleep-wake cycle lengthens dramatically to 48 hours and escapes from its normal 1:1 mode of coupling to the day-night cycle; (6) the retinohypothalamic tract, which mediates the synchronizing effects of external time cues on circadian pacemakers in the hypothalamus is intact in all manic-depressives studied to date.

Project Description:

Epidemiological evidence has emphasized the essential cyclicity of both unipolar and bipolar affective illness. Since manic-depressive illness is a periodic illness, a study of the role of biological rhythms in its pathophysiology and pathogenesis is important. This project involves longitudinal studies of behavioral, physiological, and biochemical variables in inpatients and outpatients who have relatively frequent mood cycles. Focus is on both circadian (i.e., 24-hour) and longer rhythms, requiring both intensive data collection (multiple sampling within the same day) and longitudinal data collection over time (in several patients, we have obtained daily measures for a period of many months).

Behavioral parameters studied involve both nurses' ratings and self-ratings, as well as motor activity monitoring using a novel nontelemetric solid state, computer-based device developed at NIH. Physiological parameters include EEG sleep studies, temperature, biochemical variables including urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), the urinary metabolite thought best to reflect brain norepinephrine metabolism, melatonin, cortisol, thyroid stimulating hormone (TSH), growth hormone (GH), prolactin (PL), and (less frequently) CSF amine metabolites. These data are evaluated by time series analyses in order to characterize circadian rhythms.

We can describe the findings in these studies under several headings:

1. Circadian rhythms are 24-hour cycles of biological functions which are driven by neural pacemaker cells in the hypothalamus. These rhythms have characteristic timing and waveforms which are genetically determined and homeostatically conserved. For example, body temperature rises each day to an evening peak, then declines to a late night low. We have studied the timing or phase-position of circadian rhythms in depressives compared with normals and compared with themselves in non-depressed states. We found that the timing of circadian rhythms is abnormally early in depressives. Urinary MHPG excretion is normally maximal in the late evening and minimal in the early morning. In 10 bipolar depressives who were compared with 14 controls, the pattern was shifted several hours earlier. A smaller but similar shift in the temperature rhythm was observed in the same patients. In a separate study the nightly rhythm of melatonin secretion by the pineal gland was found to be approximately one hour advanced in 5 depressives compared with 6 controls.

A few bipolar patients have been studied longitudinally through one or more manic-depressive cycles. In four cases the circadian temperature rhythm was measured. In each case the timing of the temperature rhythm became progressively earlier until the patient switched into depression; it then became progressively later until the patient switched out of depression. The range of these shifts was 5 to 12 hours. The finding of a maximal phase-advance at the switch into depression is consistent with our finding that circadian rhythms are phase-advanced in depressives compared with controls. These findings prompted us to reanalyze data in 23 studies of circadian rhythms published in the world literature. We found that in 19 of the 23 studies, depressives' circadian rhythms were 1 to 4 hours advanced compared with controls. The extensive sleep EEG studies conducted during the past 15 years in depression can also be interpreted using the phase-advance model. In normals the occurrence of rapid-eye-movement sleep (REM) is strongly coupled to the trough of the circadian temperature rhythm. Thus, REM sleep predominates in the latter half of the night's sleep, when temperature is lowest. In depression REM sleep is redistributed to earlier portions of the sleep period. This well-established REM sleep disturbance can be interpreted as a manifestation of the earlier timing of the temperature rhythm in depression. In fact, in a few patients in whom sleep EEG was studied longitudinally we found parallel state-dependent changes in the timing of the temperature rhythm and REM sleep abnormalities.

Our initial studies of temperature involved oral temperature measurements. During the past year we have used a self-contained, solid-state rectal temperature recorder which patients wear on their belt, and which monitors temperature continuously. Data from these instruments can be fed directly into a computer for subsequent plotting and analysis. We are entering into a large scale cross-sectional study of circadian temperature rhythms and EEG-monitored sleep in depressed outpatients, in order to explore the relationship between temperature and REM sleep across a broader spectrum of depressive illness.

2. We hypothesize that the phase-advance of circadian rhythms in relationship to sleep is a critical factor in establishing and maintaining the depressive state. Depriving a patient of sleep during the entire night often induces a dramatic, but short-lived, remission of the depression, suggesting that disrupting the abnormal relationship of sleep to circadian rhythms may be therapeutic. The early timing of circadian rhythms in relationship to sleep could be corrected if depressives' sleep schedule were shifted correspondingly earlier. In one patient, shifting the onset of sleep from 11 p.m. to 5 p.m. produced a remission which lasted two weeks. Following relapse, the patient's sleep was again shifted 6 hours earlier, producing a second remission.

3. The depressive circadian rhythm disturbance can be simulated in normal subjects by delaying the timing of their sleep period later than usual e.g., from 11 p.m. - 7 a.m. to 7 a.m. - 3 p.m.). By shifting sleep later relative to other circadian rhythms, the timing of other circadian rhythms is earlier relative to sleep, as in depressives. In our ongoing study, normal subjects' sleep is delayed 4, 8, and then 12 hours. After each shift, sleep EEG, temperature pattern, urinary free cortisol excretion the TSH response to TRH infusion are measured. To date, only sleep EEG data have been analyzed. With increasing delays, normals' sleep EEG patterns increasingly resembled depressives'. Neuroendocrine assays are in progress. Effects of similar experimental shifts on 24-hour plasma cortisol secretory patterns will be investigated in the coming year.

4. Since depressives' circadian rhythms are abnormally advanced, and since depression can be treated with several types of drugs, the effect of antidepressant drugs on the timing of circadian rhythms is of interest. Accordingly Dr. Wirz-Justice in our group, in collaboration with Marion Kafka and Dieter Nabor in the BPB have conducted a series of experiments designed to investigate the effects of imipramine (a tricyclic antidepressant), clorgyline (a monoamine oxidase inhibitor antidepressant) and lithium (an antidepressant in some patients) on the timing of circadian rhythms in rats entrained to a 24-hour light dark cycle. It was previously established that various brain neurotransmitter binding sites show circadian variability when animals are placed in either 12 hour light-dark conditions or in constant conditions. These include alpha, beta, dopamine, benzodiazepine, and opiate receptor sites. Imipramine and clorgyline were found to induce 4-12 hour delays in the phase-position of these rhythms in chronically treated animals. This result is in the direction predicted, if antidepressants act by delaying the abnormally advanced circadian rhythms in depressives. Lithium delayed some circadian rhythms, but more often abolished the rhythms.

5. There is a relationship between the timing of a circadian rhythm when it is entrained to the day-night cycle and the periodicity that rhythm would show if it were allowed to free-run at its own intrinsic rate in isolation from external time cues. Fast free-running rhythms entrain to the day-night cycle with an earlier phase-position than slow free-running rhythms. Thus, the early timing of circadian rhythms in depression could result from a fast free-running circadian pacemaker. Antidepressant drugs may exercise their effect by slowing this hypothesized fast-running circadian pacemaker. To explore this possibility, the effects of chronic treatment with imipramine and clorgyline on the free-running rhythm of hamsters in isolation from external time cues were studied. Animals were housed in a special laboratory in cages fitted with running wheels which were automatically monitored by a computer system designed by Wayne Rasband and Newland Morgan of the Research Services Branch. Drugs were administered at a constant rate for two weeks with implanted osmotic pumps. Both drugs slowed the free-running activity rhythms of the animals, an effect which could be expressed as a delay of the phase position of the rhythms, if they were entrained to the day-night cycle.

All of these preliminary results are consistent with the hypothesis that antidepressant drugs act by slowing the circadian pacemaker and delaying the abnormally advanced circadian rhythms in depressed patients. Two major methodological questions are under further exploration. First, are these effects specific for clinically active antidepressants? Clinically inactive isomers must thus be tested in similar experiments. Second, to what extent did the effects of maintaining the animals on a light-dark schedule confound the observed effects of drugs on neurotransmitter binding rhythms? Future studies must be carried out in such a way that all measurements before and after drug-treatment are done in darkness.

6. Definition of phase, amplitude and levels of circadian rhythms requires techniques and instruments which permit around the clock, continuous measurement of relevant variables. Longitudinal studies in patients require that these measurements be carried out day after day for months at a time. Fortunately, the development of a small solid state instrument by Theodore Colburn and his associates at the Research Services Branch has made it possible to obtain continuous longitudinal measurements of one variable, motor activity. We have studied the behavior activity-rest (sleep-wake) cycle for many months in 15 rapidly cycling manic-depressive patients. Our principal finding is that the sleep-wake cycle lengthens from its usual 24-hour period to 48 hours at the onset of mania. This consistently occurred in 11 patients, sometimes occurred in two additional patients and never occurred in two other patients. Sometimes three or more double length sleep-wake cycles were observed to occur in succession. We infer that the driving oscillator of the sleep-wake cycle markedly slowed its rate of oscillation and escaped from the primary (1:1) to the secondary (1:2) mode of entrainment to the day-night cycle. Similar lengthening of the period of the sleep-wake cycle and shift into and out of the secondary coupling mode also occurs in normal subjects during free-running in isolation experiments. In these normals, the sleep-wake cycle lengthens, escapes from 1:1 coupling with the free-running 25-hour cycles of the circadian temperature rhythm and recouples in a 1:2 mode: thus, the resulting 50-hour sleep-wake cycle locks on to every other 25-hour temperature cycle. When this occurs, normal subjects may remain awake for 30 hours or more with no subjective need for sleep. Manics, too, may stay awake the entire night with no perceived need for sleep.

In order to estimate the frequency of occurrence of double-length sleep-wake cycles in a larger population of manics, we reviewed nurses' longitudinal observations of nighttime sleep in 62 patients studied on our research ward. Over half of the patients had one or more alternate nights of total sleeplessness in association with mania (a 48-hour sleep-wake cycle would be reflected as 0 hours sleep on alternate nights). There are several possible causes of these abnormally long sleep-wake cycles during mania. One possibility is that the intrinsic rhythm of a driving oscillator of the sleep-wake cycle is abnormally slow and thus cannot consistently keep up with the 24-hour rhythm of the day-night cycle, resulting in periodic 48 hour sleep-wake cycles when the coupling shifts from 1:1 to 1:2. Another possibility is that the coupling of the sleep-wake cycle to the day-night cycle is weakened, perhaps because of a subsensitivity to external stimuli such as light which function as time cues.

With regard to the first possibility, our animal studies indicate that antidepressant drugs may slow the intrinsic rhythm of oscillators which drive circadian rhythms. This slowing could be expected to precipitate abnormal slowing of the sleep-wake oscillator, and might slow the oscillator sufficiently to induce the 48 hour sleep-wake cycle seen at the onset of mania. With regard to the second possibility, we have studied the effects of light on nocturnal melatonin secretion in four manic-depressive patients who exhibited circadian rhythm disturbances. We have previously shown that normals' melatonin is almost completely suppressed by bright artificial light. This effect is probably mediated by the same neural pathway which synchronizes circadian rhythms to light-dark cycles. The response was grossly intact in the patients, indicating either that the patients'abnormalities in rhythm entrainment are not related to a demonstrable subsensitivity or that the subsensitivity is too subtle to be shown using this technique.

Publications:

Lewy, A.J., Wehr, T.A., Gold, P. and Goodwin, F.K.: Plasma melatonin in manic depressive illness. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, Vol. 2, pp. 1173-1175.

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Wehr, T.A. and Goodwin, F.K.: Circadian rhythm desynchronization as a basis for manic depressive cycles. Psychopharmacol. Bull., in press.

Naber, D., Wirz-Justice, A., Kafka, M.S. and Wehr, T.A.: Dopamine receptor binding in rat striatum: circadian rhythm and its modification by chronic imipramine. Psychopharm., in press.

Wirz-Justice, A., Wehr, T.A., Goodwin, F.K., Kafka, M.S., Naber, D., Marangos, P.J. and Campbell, I.C.: The effect of antidepressant drugs on circadian rhythms in behavior and brain receptors. Psychopharmacol. Bull., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01851-03 CP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Melatonin Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3.1	1.1	2.0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The role of the pineal gland and its hormone, melatonin, has been difficult to define in humans, because the circulating levels of melatonin have been too low to measure. In collaboration with Dr. S. Markey of the LCS, we have developed a highly sensitive and specific gas chromatographic-mass spectrometric (GC-MS) assay which requires less than 1 ml of human plasma, CSF, or urine. We are currently investigating: (1) the circadian pattern of secretion of melatonin in normal individuals and its alteration in a variety of disease states (such as depression, mania, and blindness); (2) the relationship between melatonin secretion and other circadian rhythms; (3) the effect of drugs on melatonin secretion; (4) the effect of different intensities of light on melatonin secretion; and (5) the use of basal and isoproterenol-stimulated melatonin levels to assess beta-adrenergic receptor function. We have found a phase shift of the usual circadian secretory pattern in affectively-ill patients and a significantly disrupted pattern in blind individuals. Tricyclic antidepressants produce changes in melatonin secretion which are consistent with alterations in both pre- and post-synaptic beta-adrenergic functioning. There is a "dose-response" relationship between the intensity of light administered in the early morning hours and the resultant suppression of melatonin secretion.

Project Description:

Objectives: The pineal gland is of considerable interest not only because its basic role in endocrine function in man is unknown, but also because it provides an *in vivo* marker for the biological clock regulating the activity-rest cycle (see below), as well as being a measure of the activity of a well-characterized beta-adrenergic receptor. In man, plasma levels of melatonin are extremely low, on the order of 1-2 picogram/ml during the day, not quantifiable by previous techniques. Dr. Lewy has developed a gas chromatographic-mass spectrometric (GC-MS) method for the measurement of melatonin in collaboration with Dr. Sanford P. Markey of the LCS. This technique has the requisite specificity and accuracy to measure melatonin in humans in quantities as little as 1 cc of plasma. In addition to affective illness, other diseases of interest include those involving the thyroid and adrenal, precocious and delayed onset of puberty, infertility, hypertension, malignancy, including cancer of the breast and ovary.

The neurotransmitter norepinephrine is the normal physiologic stimulant of the pineal gland's secretion of melatonin. Attention has been focused on this neurotransmitter in conjunction with affective disorders and other psychiatric diseases. We are studying the effects of various psychotropic medications, including tricyclic antidepressants, lithium, neuroleptics, and MAO inhibitors, both acutely and chronically. Many of these medications have been thought to induce changes in β-adrenergic receptors. It is now possible to document *in vivo* these changes in humans.

Methods: Although radioimmunoassays (RIAs) and bioassays were used for measurement of human plasma melatonin, problems with insufficient sensitivity, specificity or efficiency have severely limited their use for routine analysis. Whereas theoretically GC-MS is the most specific of these techniques, attempts to apply it to melatonin analysis have also failed because of insufficient sensitivity. Use of a new technique, negative chemical ionization, and improved chromatographic and extraction procedures, led to a GC-MS method that has the requisite sensitivity, specificity and efficiency for the routine analysis of melatonin in human plasma, CSF, and urine. Less than 1 ml of sample is required, thus, permitting wide flexibility of experimental design and repeated and frequent measurements. Daytime concentrations as low as 1-2 picogram/ml and night-time values as high as 100 picograms/ml can be measured, yielding a 20-50 fold range. The other available assays (RIAs and bioassays) generally report higher values and a lower range, probably because of their lack of specificity.

Major Findings: We have concluded our first investigation of melatonin in manic-depressive illness. Humans display the predicted nighttime rise and daytime decrease in melatonin secretion, as do other subhuman species. As reported normal controls secrete melatonin to a maximum 25-100 picograms/cc during the night. Manic patients, however, secrete significantly more melatonin during the night than do normals or depressed patients. Bipolar depressed patients secrete less melatonin than do normals or manic patients. These results are consistent with the catecholamine hypothesis of affective disorder, which states that noradrenergic activity is increased in mania and decreased in depression.

The acrophase of melatonin secretion is earliest in manic patients and latest in normal subjects; this is consistent with the phase-advances in temperature and 3-methoxy-4-hydroxy-phenylglycol (MHPG) in affectively ill patients (See 201 MH 00450-06 CP).

We are interested in the effect of propranolol, the β-adrenergic receptor blocker, in the treatment of manic-depressive illness. Uncontrolled studies of propranolol in various psychiatric disorders have suggested positive effects in states associated with hyperactivity, such as mania and agitated depression. We have extended these studies using a double-blind paradigm. While the therapeutic efficacy of propranolol in treating mania and depression has been quite variable, we have noted a rather clear-cut effect of propranolol on the frequency of affective episodes in rapid-cycling bipolar patients: three patients have shown much more rapid cycling in association with propranolol administration and withdrawal. Assays of plasma melatonin in such instances may help elucidate the relationship between beta-adrenergic blockade and the observed clinical events.

As a sequel to our studies of the effects of tricyclic antidepressant administration on melatonin secretion in patients, we have initiated studies of melatonin secretion in normal volunteers during acute and chronic administration of tricyclics. Data analysis is underway.

Using the melatonin assay, we have also studied the effects of the tricyclic antidepressant desmethylimipramine (DMI) on noradrenergic function in animals. Overall output of the noradrenergic system controlling melatonin secretion can be assessed by measuring the hormone at night. Changes in postsynaptic receptor function can be delineated by measuring the response of melatonin to isoproterenol injection during the day. The state of presynaptic function can therefore be estimated by subtraction. We have found that DMI causes a 20% reduction in isoproterenol stimulation of melatonin during the day, implying a 20% reduction in postsynaptic β-adrenergic receptor function. However, the nighttime melatonin secretion is reduced by more than 50%, indicating that some reduction in presynaptic function is also associated with DMI treatment. These studies complement other ongoing animal studies in the Branch regarding drug-induced changes in neurotransmitter receptor sites.

In animals, artificial light of any intensity blocks melatonin secretion abruptly and completely. In humans, however, we have previously reported that artificial light of the approximate intensity of sunlight is required to suppress melatonin secretion; artificial light of normal intensity is not sufficient. Extensions of these studies in normal volunteers during the past year have revealed that a "dose-response" relationship exists between the intensity of light and the degree of suppression of melatonin secretion. We are currently studying the "dose-response" relationship in affectively-ill patients.

In collaboration with Dr. W. Mendelson of the Sleep Lab (BPB), we studied the stages of sleep in relation to melatonin secretion. Contrary to our initial preliminary findings there appears to be no correlation between bursts of melatonin during the night and the stages of sleep.

We are collaborating with the National Eye Institute with Dr. Elmer Ballentine and Dr. David Newsome. We are looking at melatonin levels in patients with retinitis pigmentosa. This disease has no known cause; and because of the link with a pigmenting agent such as melatonin, it is possible that the explanation for this disease may be in understanding melatonin levels. We are also studying the melatonin diurnal patterns of secretion in blind patients, and the light intensity necessary for suppression of melatonin secretion in patients with different types of impaired light perception. Preliminary results indicate that blind individuals have grossly abnormal secretory patterns.

Publications:

Lewy, A.J., Wehr, T.A., and Goodwin, F.K.: Melatonin secretion in manic-depressive illness. Proceedings of the Second World Congress of Biological Psychiatry. Amsterdam, Elsevier/North Holland, 1979, Vol. 2, pp. 563-565.

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Mendelson, W.B., Gillin, J.C., Dawson, S.D., Lewy, A.J., and Wyatt, R.J.: Effects of melatonin and propranolol on sleep of the cat. Brain Res. in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00447-11 CP

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October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Amine Neurotransmitters and Metabolites in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Amine neurotransmitters and their metabolites are studied in blood, cerebrospinal fluid (CSF), and urine to assess neurochemical alterations which may be associated with clinical diagnoses, clinical states, particular behavioral or psychopathological dimensions, or treatment with drugs. Because of both methodological advances in assays for low concentrations of metabolites and problems associated with the probenecid technique, CSF studies have focussed increasingly on baseline (non-probenecid) lumbar punctures. During the past year sources of variance in urinary MHPG have been addressed in several methodological studies. Previously-reported diagnostic group differences and state-related changes in urinary MHPG have been confirmed. A variety of personality and behavioral variables have been associated with specific CSF and urinary metabolites. Drug response prediction studies based on pre-treatment metabolite levels continue. The completed desmethylimipramine/zimelidine crossover trial demonstrates specific in vivo CSF metabolite changes corresponding to in vitro effects on reuptake (i.e., the noradrenergic tricyclic selectively decreases CSF MHPG, while the serotonergic tetracyclic selectively decreases CSF 5-HIAA); however, both agents decrease urinary MHPG, raising further questions about the meaning of the urinary measure.

PHS-6040
(Rev. 10-76)

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Giovanni Muscettola	Assoc. Prof.	Univ. of Naples

Project Description:

The purpose of this project is the direct assessment of the functional state of brain neurotransmitter amines in patients and normal individuals by assay of levels of the amines and their major metabolites in plasma, in urine, and in the cerebrospinal fluid (CSF). The metabolites under study are 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin; homovanillic acid (HVA), the major metabolite of dopamine; and 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3-methoxy-4-hydroxyphenylacetic acid (VMA), the major metabolites of norepinephrine.

The Chief, CPB, serves as the coordinator for CSF studies in the Division; the Unit on Neurochemistry, CPB provides sample processing and assays (the latter in collaboration with Edna Gordon and Michael Ebert of the LCS); this Branch also provides central data storage and analysis for CSF results. The major collaborating groups for the CSF studies are the Sections on Psychobiology (Dr. Post) and Neuropharmacology (Dr. van Kammen) of the Biological Psychiatry Branch and the Clinical Neuropharmacology Branch (Dr. Murphy). Studies from the Clinical Psychobiology Branch are reported in detail here. Studies from collaborating groups are reported further details are found briefly in the annual reports of the groups involved.

Methodological Issues

Cerebrospinal fluid studies have previously involved both "baseline" lumbar punctures (LP's) and LP's following the administration of probenecid, an organic acid which competitively inhibits the active transport of acid metabolites from CSF. However, completed studies suggest that there are major sources of variance attributable to the probenecid methodology: the incidence of nausea and vomiting is related to CSF probenecid levels and is independent of dose; weight changes during hospitalization affect CSF probenecid levels; CSF metabolite accumulation is significantly correlated with CSF probenecid levels, but the strength of the correlation varies markedly among diagnostic groups; and medications such as lithium affect the CSF metabolite-probenecid correlation.

Previously, one of the prime justifications for the probenecid technique was the high coefficient of variation of metabolite levels in baseline studies when fluorometric assays were used. In contrast, more recently available gas chromatographic-mass spectrometric (GC-MS) assays, performed by Edna Gordon, Michael Ebert, and David Jimerson, produce relatively sensitive, specific, and reproducible results even at the low metabolite concentrations found in non-probenecid CSF studies. Because of this, we have increasingly focussed on CSF studies without the probenecid methodology.

This year, the Branch has placed a high priority on the development of a high pressure liquid chromatographic (HPLC) method for assaying CSF metabolites. When fully developed and verified against the GC-MS assay, the HPLC assay will provide an accurate assay, available in the CPB's own laboratory.

A study of the comparability of three different assays for urinary MHPG has been completed. The NIMH modification of the original GC assay provides a radioactive tracer which enables the researcher to correct for varying degrees of hydrolysis and recovery. The within-run coefficient of variation (CV) for duplicates is .04; the between-run CV is .10. The modified method shows a .83 correlation with the original GC method, and a .98 correlation with the GC-MS method of Gordon and Oliver, suggesting that the modification represents a significant advance over the original GC assay for urinary MHPG. For reasons which are poorly understood, the modified method results in values of MHPG which are about 20% higher than values using GC-MS. The low CV's and the high correlation with GC-MS suggests that interindividual comparisons done within a given laboratory using this method will be reliable; however, the lower correlation with the unmodified method and the difference in absolute values from the GC-MS method suggest that comparisons across laboratories and assays are fraught with difficulties.

Urinary MHPG appears to be quite stable over at least a two-year period when preserved with sodium metabisulfite and stored at -20°C. No consistent directional change in MHPG was observed on reassay after two years; the interassay CV was .14.

Monoamine Metabolites, Age, Sex, and Activity

Several investigations of potentially confounding variables such as age, sex, and activity suggest that the effects of these variables must be assessed separately in different populations. We have previously noted that the relationship between CSF probenecid levels and metabolite accumulations is strong and highly significant in bipolar patients, is weaker in unipolar patients, and is absent in schizophrenics and in patients on lithium. Age shows a weak positive correlation with urinary MHPG in depressed patients but not in normal controls. Among depressed patients, a sex difference in MHPG excretion is apparent, with males excreting 20% more than females ($p < .005$); no such sex effect is seen in normal controls. Activity (as measured by the solid-state portable activity monitor) shows a correlation of $r = 0.55$ with urinary MHPG in depressed patients, but a much lower, non-significant correlation in manic patients and normals. Coupled with other previously reported studies from this Branch these data suggest that levels of monoamine metabolites in normals are relatively uninfluenced by many of the factors influencing levels in many affectively-ill patients. Whether this observation is of pathophysiological significance remains to be determined.

Relationships Among Monoamine Metabolites

In a study of 27 patients, no relationship was found between urinary and CSF MHPG levels, suggesting that these values represent different aspects of NE metabolism. In addition, Dr. Jimerson's studies of plasma MHPG in normal volunteers (reported in Project Z01 MH 00070-07 BO) suggest a high correlation between plasma MHPG and CSF MHPG ($r = 0.70$) and no correlation between plasma MHPG and urinary MHPG.

However, the relationships among levels of MHPG in these different fluids and the relationships of these levels to central NE metabolism are still quite uncertain.

For example, in studies by our Branch and Dr. Post's Unit, patients who are manic compared with those who are depressed show higher levels of CSF NE, plasma NE, plasma MHPG, and urinary MHPG, while CSF MHPG is not significantly elevated. In contrast, changes in CSF MHPG most closely correlate with differing in vivo effects of desmethylimipramine and zimelidine on NE metabolism, while urinary MHPG does not distinguish between these drugs.

Given the current uncertainty about the origins and significance of MA's and MA metabolites, we have elected to continue studies of blood, urine, and CSF to further delineate the complex interrelattionships among these measures of noradrenergic functioning.

Monoamine Metabolites, Clinical State, and Diagnosis

In collaboration with other NIMH units, we have continued the exploration of CSF amine metabolites within and between various clinical states and diagnostic groups (affective illness, schizophrenia, and schizoaffective disorders) with the emphasis on primary affective illness.

Our Branch has completed a replication of our previous urinary MHPG studies in affective illness. Seventy depressed patients, thirteen manic patients, and twenty-seven normals were studied. Urinary MHPG in bipolar patients was significantly lower in depression than in mania (averaging 20% lower, $p < .01$). Bipolar I depressed patients had significantly lower urinary MHPG excretion than either unipolar depressed patients ($p < .05$) or normals ($p < .02$), with bipolars averaging 20% lower than both comparison groups. These data are consistent with most reports in the literature, as described in Goodwin and Potter's review article on noradrenergic function in affective illness.

However, studies of MA metabolism have expanded beyond the traditional focus on patients with primary affective disorder or schizophrenia to include patients with atypical depressions, character disorders, and alcoholism, as well as normal individuals. By designing studies which cross diagnostic boundaries, we hope to identify correlates of specific psychopathological dimensions. For example, alcoholism has been associated with abnormal serotonin metabolism. A recent report from our Branch (Rosenthal, et al.) finds that patients with primary affective disorder who have a family history of alcoholism have a lower probenecid-induced level of 5-HIAA in CSF. Similarly, serotonergic and noradrenergic mechanisms have been associated with impulse control. Studies in normal volunteers (Dr. Ballenger) and character disordered individuals (Dr. Brown) suggest that aggressive, violent behavior is associated with high CSF MHPG and probably with low CSF 5-HIAA in both populations; thus, specific pathophysiological mechanisms may be significant across diagnostic groups.

Monoamine Metabolites as Predictors of Drug Responses

A series of reports from this group relating initial metabolite levels to treatment response have appeared over the last 10 years, suggesting that low pretreatment levels of a metabolite are associated with good antidepressant response to drugs whose predominant initial action is to enhance the activity of the neurotransmitter in question. Our continuing inpatient studies now show only a trend for responders to desmethylimipramine (DMI, a predominantly noradrenergic tricyclic) to have low pretreatment urinary MHPG levels; the significance of this finding is unclear, as the population studied contained a greater proportion of either seriously-ill, treatment refractory patients or rapid cycling patients. Studies with a broader spectrum of depressed patients are indicated.

Alterations in Monoamine Metabolites Produced by Drugs and ECT

The desmethylimipramine/zimelidine crossover study has been completed. Preliminary results of CSF and urinary MA metabolites suggest the *in vitro* specificity of each drug for blocking noradrenaline reuptake and serotonin reuptake respectively is mirrored in relatively specific effects on CSF metabolites, but not on urinary metabolites. Desmethylimipramine selectively decreased CSF MHPG and had little effect on CSF 5-HIAA, whereas zimelidine decreased CSF 5-HIAA and had little effect on CSF MHPG. Both drugs decreased urinary MHPG excretion, as previously noted. In addition to the suggestion that CSF MHPG may correlate more closely with drug effects than urinary MHPG, the results indicate that non-probenecid LP's may be as useful as probenecid LP's in identifying drug effects, provided sensitive GC-MS assays are employed to measure metabolite levels. One further finding of interest is that the degree of change in metabolite levels bare no relationship to either global clinical response or to drug effects on circadian patterns of motor activity.

Two studies using the probenecid method to assess metabolite changes induced by ECT and lithium suggest that these treatments produce similar changes in CSF metabolites. CSF 5-HIAA is significantly decreased by both treatments; however, neither initial 5-HIAA levels nor the decrease in 5-HIAA were related to clinical response. CSF HVA was not significantly altered by either treatment. There was, however, a strong relationship between pretreatment HVA levels and subsequent changes during treatment, with high HVA levels decreasing and low HVA levels increasing. However, again neither the pretreatment levels nor the changes during treatment were related to clinical response.

Further Directions

The availability of a brief inpatient evaluation program will enable us to examine MA metabolites in a broader spectrum of patients. Of particular interest is whether patients with non-endogenous depressive syndromes have high urinary MHPG (as suggested by Schildkraut) and whether individuals with disorders of affect and impulse control (borderline personalities) demonstrate low CSF 5-HIAA (as suggested by our previous studies of sociopaths). In addition, MA correlates of response to monoamine oxidase inhibitors will be examined.

Other current interests under investigation include the study of a variety of neuropeptides and hormones in CSF. A GC-MS assay for melatonin (see report Z01 MH 01851-01 CP) permits the reliable measurement of the minute quantities of melatonin found in CSF. Assays for vasopressin in CSF may provide an avenue for exploring hypotheses linking vasopressin with primary affective disorder. Finally, the availability of results from Dr. Gold's integrated neuroendocrine test will allow closer examination of the relationships between CSF metabolites and neuroendocrine challenge tests as markers of central neurotransmitter activity (see report Z01 MH 00452-05 CP).

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Post, R.M., Ballenger, J.C., Hare, T.A., Goodwin, F.K., Lake, C.R., Jimerson, D.C., and Bunney, W.E., Jr.: Cerebrospinal fluid GABA in normals and patients with affective disorders. Brain Res. Bull., in press.

Ebert, M.H., Cowdry, R.W., Post, R.M., and Goodwin, F.K.: CSF probenecid level in the oral probenecid test. Psychopharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01850-03 CP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antidepressants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Irwin J. Kopin	Chief	LCS/NIMH
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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	2.5	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tricyclic antidepressants (TCAs) are extensively used in clinical practice without an adequate understanding of their clinical pharmacology. New and continuing studies to provide rational guidelines for the use of TCAs include: (1) the successful application of single dose pharmacokinetic predictions in normal volunteers and patients of all ages, including the aged (>80 years); (2) the influence of age (7-90 years) on plasma levels of desipramine (DMI); (3) an animal model for pharmacokinetics of DMI its active hydroxylated metabolite; (4) a pharmacokinetic explanation for specificity of action of the experimental antidepressant and serotonin-uptake inhibitor, zimelidine (ZIM), in man; (5) the use of cerebrospinal fluid (CSF) drug level as the best index of free drug; (6) the effects of DMI on norepinephrine (NE) its deaminated metabolites, pulse and blood pressure; effects consistent with the single mechanism of NE uptake inhibition; (7) more detailed studies of neuroendocrine effects of DMI and ZIM, demonstrating that both have effects which could reflect altered serotonergic function despite their in vitro biochemical differences (8) a study of the effects of DMI and ZIM on the activity-rest cycle, showing similar behavioral effects despite different effects on NE metabolism.

Project Description:

Studies of the clinical and pharmacological effects of antidepressant medication have been expanded during the past year, with the continued participation of Dr. Helena Calil, a Visiting Fellow, and Dr. Richard Ross, a PRAT Fellow. This year a further increase in project staffing has occurred: Dr. Kitanaka joined the Branch as a Fogarty Visiting Fellow and is working both on pharmacokinetic studies of hydroxylated metabolites in rats and the chronopharmacology of TCAs in man using the HPLC assay; and Dr. Markku Linnoila, an experienced M.D.-Ph.D. clinical psychopharmacologist, has joined the program on an IPA as of July 1, 1980.

Three distinct areas of study have been pursued: (1) the development of reliable assays for antidepressants, (2) the use of pharmacokinetics to provide maximally effective utilization of drugs, and (3) the study of clinical and biochemical effects of specific antidepressants in patients and normals as a means of studying the biochemistry of major affective disorders.

Assay Development

The application of an established gas chromatographic-mass spectrographic (GC-MS) assay for tissue concentrations of tricyclic antidepressants (TCAs) has made expanded pharmacokinetic and pharmacodynamic studies possible. In addition, we have established and validated a high pressure liquid chromatograph (HPLC) assay for TCAs and their hydroxylated metabolites with the assistance of Dr. Britt Mellstrom from the Karolinska Institute, Stockholm, Sweden. Routine analysis of both patient and animal tissue samples is now done in our own laboratory with this assay. GC quantitation of the experimental tetracyclic antidepressant, zimelidine, and its active metabolite, norzimelidine, has permitted parallel studies with this biochemically contrasting drug.

Pharmacokinetic Studies

(1) On the basis of our success using single oral doses to predict ultimate steady-state concentrations (Z01 MH 01850-01 CP) we have extended this test to clinical studies of desipramine in normal volunteers and geriatric nursing home patients (the latter in collaboration with Dr. Neal Cutler). The intravenous administration of desipramine for single dose studies has been well tolerated; the resulting prediction of steady-state levels of DMI has provided a means of choosing a treatment dosage which avoids excessive plasma desipramine levels.

(2) The study of steady-state concentrations of desipramine in hospitalized normal volunteers has shown that this population of young males and females (age 21 to 25) is pharmacokinetically undistinguishable from our patient population. Moreover, we find no evidence of altered pharmacokinetics of desipramine in a nursing home population with a mean age of 80 years. Evaluation of hydroxylated desipramine in this population is currently underway.

(3) Studies on single dose and steady-state pharmacokinetics of TCAs in rats demonstrate that earlier measurements of half-lives have employed a too simple model and ignored the true terminal disappearance curve. Initial studies with in vivo administration of the active hydroxylated metabolite of desipramine show a very rapid half-life. With such a short half-life, steady-state would only be achieved using a constant infusion of the hydroxylated metabolite, a situation which in fact occurs with administration of imipramine or desipramine, since the desipramine present in the blood is continually converted to its active hydroxylated metabolite.

(4) As further data on hydroxylated metabolites of TCAs become available, there is a suggestion that approximately 5% of the population are essentially non-hydroxylators. This is of considerable pharmacogenetic interest since a similar deficiency in the hydroxylation of another potent drug, debrisoquine, has recently been identified by two different groups.

(5) The usefulness of CSF levels of drugs as the best measure of free drug has been further supported by the finding that in vitro equilibrium dialysis gives an inaccurate (and excessive) estimate of free norzimelidine in man. These CSF studies of drug levels have thus far only been performed here and at the Karolinska Institute.

Clinical and Biochemical Effects of Antidepressants

(1) Ten patients have now completed a unique double-blind cross-over trial in which an experimental antidepressant (zimelidine), specific for serotonin uptake inhibition, is compared with an established antidepressant (desipramine) specific for norepinephrine uptake inhibition. The preliminary finding that zimelidine does not precipitate mania or rapid cycles in bipolar depressed patients (as contrasted to desipramine) has been extended; in addition we have provided further evidence of a lack of overall difference in the antidepressant efficacy of the two drugs, despite differential effects on MHPG and 5-hydroxy-indole acetic acid (5HIAA) in the CSF (see Z01 MH 00447-10 CP).

(2) In volunteers the effects of desipramine on urinary and plasma MHPG and plasma NE are consistent with a primary drug action as an inhibitor of the reuptake of released NE: NE itself is increased, while its deaminated metabolites are decreased. These data do not provide support for the assumption that decreases in MHPG reflect decreased turnover of NE.

(3) More extended studies on the acute and chronic effects of desipramine on cardiovascular function and changes in NE release and metabolism are underway. Within the narrowly-controlled range of desipramine studied, no relationship between NE, its metabolites, and caridovascular effects has emerged.

(4) Although initial studies of the effects of DMI and ZIM on neuroendocrine function showed no consistent pattern (Z01 MH 00452-04), more extensive data from a larger number of patients have revealed more clear cut results. Following administration of DMI there is a state dependent effect on growth hormone (GH); non-depressed subjects show acute increases of GH whereas depressed patients show no alteration of GH. Of particular interest is the finding that acute DMI also stimulates prolactin release which could be interpreted as a serotonergic effect. Serotonin has been implicated as stimulatory to prolactin and in animals DMI may increase responsiveness of serotonin receptors. On the other hand, in the same patients, neither acute nor chronic ZIM increased prolactin. Finally, chronic ZIM increases vasopressin in the CSF, an increase that is consistent with a postulated serotonergic role in vasopressin release.

(5) One of the most interesting and surprising findings is that both ZIM and DMI produced similar and marked effects in the rest-activity cycle of depressed patients independent of their effect on mood. With both drugs, total activity increased while "night-time" activity (midnight to 7 a.m.) dramatically decreased. This may provide a means of ruling out a primary role of NE in the control of the rest-activity cycle since DMI and ZIM have differential effects on this neurotransmitter.

(6) We have shown that norzimelidine predominates over zimelidine in the CSF by a ratio of 7 to 2, so that levels of the metabolite norzimelidine are probably of much greater clinical importance than levels of the parent compound zimelidine.

(7) We have extended our initial studies of the relationship between zimelidine dose and plasma levels of norzimelidine, and continue to observe a close relationship between dose and blood levels of the major active compound, a situation quite unlike that found with the TCAs.

Publications:

Potter, W.Z., Calil, H.M., Zavadil, A.P., Jusko, W.J., Sutfin, T., Rapoport, J.L., and Goodwin, F.K.: Steady-state concentration of hydroxylated metabolites of tricyclic antidepressants in patients: relationship to clinical effect. Psychopharmacol. Bull. 16(2): 32-34, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00452-05 CP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neuroendocrine Studies of Major Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Daniel van Kammen	Chief, Section on Neuropsychopharm	BPB/NIMH
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	Michael Ebert	Chief, Section on Exp. Ther.	LCS/NIMH
	Seymour Reichlin	Chief, Division of Endocrinol.	Tufts Univ.
	Detlev Ganten	Prof., Dept. of Pharmacol.	U. of Heidelberg
	Gregory Brown	Chief, Dept. of Neurosciences	McMaster Univ.

COOPERATING UNITS (if any)
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TOTAL MANYEARS: 3.1	PROFESSIONAL: 1.5	OTHER: 1.6

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hormones of the hypothalamic pituitary axis (HP) exert direct behavioral effects, either by activation of specific receptors in brain or by modulation of central neurotransmitter systems. The synthesis and release of HP hormones is partially under the control of the monoaminergic neurotransmitters and is influenced by psychoative agents. Current studies concentrate on comprehensive tests of HP function and on the measurement and administration of specific behaviorally active peptides or peptide analogs. Major findings this year are: cerebrospinal fluid (CSF) vasopressin (VP) is significantly lower in drug-free depressed patients compared with controls. Desamino-8-D arginine vasopressin (DDAVP), a VP analog, enhances cognition in normals as well as in patients with affective illness. Patients with anorexia nervosa show a state independent increase in the CSF/plasma VP ratio and grossly disturbed osmoregulation of VP secretion. I.V. methadone infusion causes sustained suppression of plasma cortisol in depressed patients. The growth hormone and prolactin responses to I.V. arginine infusion are significantly greater in unipolar compared with bipolar depressed patients or controls.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Gary Robertson	Professor of Medicine	Univ. of Chicago
Samuel Yen	Chief, Dept. of Reproductive Endocrinology	UCSD

Project Description:

Several aspects of the symptom complex of the functional psychoses, particularly affective illness, suggest hypothalamic dysfunction. Thus, patients with depression or mania often show disturbances in sleep, altered energy levels, changes in appetite and libido, diurnal variation in symptoms, alterations in the consolidation of memory traces, and changes in reproductive function such as amenorrhea. Interest in the hypothalamic-pituitary (HP) axis has been further stimulated by recent findings that the monoamine neurotransmitters modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones. Thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, these peptides and hormones may have specific receptors in brain and have been shown to affect the brain neurotransmitter systems. Several of these hormones have also been shown to be responsible for specific behavioral effects.

Several neuroendocrine strategies are currently in use: (1) direct measurement in CSF and in plasma of behaviorally active peptides following stimulation according to verified stimulation paradigms; (2) administration of various hypothalamic releasing factors to test responses of the HP axis, and to elucidate patterns of monoaminergic disturbance and neuroendocrine dysfunction in patients with affective illness, schizophrenia, and anorexia nervosa; (3) psychoactive drug effects on HP function and on the levels of behaviorally active peptides; (4) assessment of the temporal organization of neuroendocrine function; and (5) assessment of the relationship between neuroendocrine function and sleep.

Because of limited laboratory support personnel and the large volume of assays required to complete this neuroendocrine project, the Unit on Neuroendocrinology maintains collaborative and contractual arrangement with several university research facilities for neuroendocrine assays. We are fortunate to collaborate with scientists of outstanding caliber, including Dr. Reymour Reichlin, Dr. Samuel Yen, Dr. Gregory Brown, Dr. Gary Robertson, and Dr. Detlev Ganten.

1. Studies of behaviorally active central nervous system (CNS) peptides:

a. The role of the CNS peptide VP is currently under study, particularly in patients with affective illness. In a major published review, we formulated an hypothesis suggesting a role for vasopressin in the regulation of affect in man. The review stressed the functional relationship between vasopressin and many aspects of the symptom complex of affective illness, including alterations in the consolidation of memory, modulation of pain sensitivity, synchronization of biological rhythms, timing and quality of REM sleep, and the regulation of fluid and electrolyte balance. We concluded that the majority of the available data in experimental animals suggested, as a first approximation, that central vasopressin function is relatively augmented in mania compared with depression.

Our first quantitative studies of vasopressin function in man involved measurement of CSF vasopressin levels in both patients and control subjects. The CSF seems to be a major communication pathway for conveying VP to various areas of the brain where it may modulate cell aggregates controlling one or more physiological process. Findings in untreated manic-depressive subjects show that CSF VP is significantly higher in manic patients than in depressed patients or controls. We have now demonstrated that, in addition, CSF VP is significantly lower in a group of drug free unipolar and bipolar depressed patients compared with controls.

An osmoreceptor located within the hypothalamus is mainly responsible for the regulation of plasma vasopressin levels. The centrally mediated set point to osmotic stimuli reflects the cumulative effects of various inputs, either peptidergic (e.g., endorphinergic) or monoaminergic, that regulate osmoreceptor function. We have evaluated osmoreceptor function in patients and controls by measuring the VP response to a sustained, carefully timed I.V. infusion of hypertonic saline. It has been shown that a linear relationship exists between VP levels and increasing plasma osmolality if hypertonic saline is infused at the rate of .05 ml/kg/min. We have demonstrated that the correlation between VP and osmolality (sensitivity) and the threshold for VP secretion is reproducible within a given individual and is thus, a reliable biologic marker when followed longitudinally. It has also been demonstrated that the threshold and sensitivity for VP secretion seem to be heritable, since they are virtually identical in homozygous twins. In continuing studies of patients with affective illness, patients in the manic state show greater sensitivity of VP responses to hypertonic saline than when they are depressed. This finding, in association with elevated CSF VP in mania compared with depression, is compatible with our initial hypothesis of relatively augmented VP function in mania compared with depression, and has now been validated with larger numbers of affectively ill patients.

To continue our study of the possible relationship of VP to affective illness, we have extended the double-blind placebo controlled trial of the VP analog DDAVP to additional patients with major affective illness. The effect of DDAVP and other VP analogs is also being studied in patients with dementia and schizophrenia, and in control subjects. Preliminary data show that DDAVP induces highly significant and consistent improvements in measures designed to quantify the formation, encoding, and organization of long-term trace events in memory. Some patients also showed significant amelioration of depressive and psychotic symptomatology during DDAVP treatment. These responses were less reliable than the cognitive responses. DDAVP also tended to enhance cognition in a group of college age control subjects. Among both patients and controls, the structure of cognitive changes following DDAVP treatment is analogous to that seen when patients recover from depression and resemble those which follow amphetamine stimulation. Specifically, the formation of long-term (relatively permanent) trace events in memory and their effects on encoding and organization are altered more than those processes that are either attentional or measures of immediate or short-term memory.

In collaboration with Dr. Walter Kaye and Dr. Michael Ebert of the LCS we have evaluated VP function in patients with anorexia nervosa. Previous work suggested that some patients with anorexia nervosa show the syndrome of partial central diabetes insipidus, although this finding was previously demonstrated only by indirect means. Both CSF VP and the plasma VP response to hypertonic saline were evaluated. Drug free patients with anorexia nervosa show a state independent increase in the CSF/Plasma AVP ratio. Thus, whereas the normal CSF/Plasma VP ratio is 1 to 2, the ratio in patients with anorexia nervosa is 3 to 1. In those patients whose weight has returned to normal for more than one year, the CSF/Plasma ratio is even greater than 3 to 1. The significance of this increased activity of the central VP secretory pathway in patients with anorexia nervosa is unclear; some animal data do suggest that VP may be involved in modulating appetitive phenomenon. Patients with anorexia nervosa also show a state independent defect in the precision of the VP response to hypertonic saline, namely, the significant positive correlation normally seen between VP and osmolality during saline infusion is absent. The only analogous situation in clinical medicine previously reported is that of complete neoplastic ablation of the osmoreceptor resulting in chaotic VP responses to hypertonic stimuli.

b. CSF levels of angiotensin I, angiotensin II, and angiotensinogen continue to be studied in collaboration with Dr. Detlev Ganten. Angiotensin is a central peptide that modulates fluid and electrolyte homeostasis. It influences learning processes in experimental animals, and is involved in perception of pain and pleasure. Levels of the enzyme that catalyzes the conversion of angiotensin I to angiotensin II have been reported to be deficient in the brains of schizophrenic subjects studied post-mortem. Preliminary data in our schizophrenic subjects are compatible with this finding; we show elevated levels of CSF angiotensin I in these patients compared with normals.

c. CSF somatostatin levels were studied in collaboration with Dr. Robert Post, Dr. David Rubinow, and Dr. Seymour Reichlin. The data so far show significantly elevated CSF somatostatin levels in mania compared with depression. In addition, there is a trend for somatostatin levels to be lower in bipolar depressed patients compared with unipolar depressed subjects, with levels in depressed patients being lower than controls, analogous to findings concerning the pattern of urinary MHPG excretion. This similarity between CSF somatostatin and urinary MHPG secretion is of interest since norepinephrine is considered to be excitatory to somatostatin secretion. The augmented CSF somatostatin levels in unipolar compared with bipolar depressed patients is analogous to other neuroendocrine differences between unipolar and bipolar subjects, such as our previously reported findings that the TSH response to TRH is blunted in unipolar compared with bipolar depressed subjects.

d. Neuroendocrine effects of opiate agonists and antagonists: Evidence from experimental animals suggests that the endorphinergic system plays an important role in the modulation of a variety of neuroendocrine systems, including vasopressin, prolactin, growth hormone, the gonadotropins, and cortisol. However, in patients large doses of naloxone do not affect baseline LH, FSH, prolactin, or growth hormone levels, the VP response to hypertonic saline, or the prolactin and TSH response to TRH infusion. Acute methadone and β-endorphin infusion in depressed patients causes marked prolactin stimulation and moderate growth hormone release.

Cortisol was markedly suppressed by both methadone and beta-endorphin infusion. Moreover methadone, that has an extended half life, induced suppression of cortisol in depressed patients for at least twenty-four hours. It may thus be possible to devise a methadone suppression test to evaluate the set point for feedback inhibition exerted by opiates on the hypothalamic pituitary adrenal axis, analogous to the dexamethasone suppression test, which evaluates the set point for feedback inhibition exerted by corticosteroids on hypothalamic pituitary adrenal (HPA) function.

2. Dynamic tests of monoaminergically regulated HP systems:

a. We continue to evaluate the response to a three hour infusion of dopamine (DA) in patients with affective illness, schizophrenia, anorexia nervosa, and control subjects. In collaboration with Dr. Gregory Brown, we have previously shown in normals a significant LH decrement during DA infusion followed by a rebound at the end of the infusion; the LH response reflects the functional activity of hypothalamic DA receptors, while the prolactin response reflects the status of both hypothalamic and peripheral (pituitary) DA receptors.

Drug free psychotic schizophrenic patients show significantly greater LH suppression during DA infusion with absent LH rebound following the infusion, while drug free manics show a paradoxical LH rise during DA infusion. The data on schizophrenic patients suggest hypersensitivity of some hypothalamic DA receptors; the paradoxical LH response in manic patients suggests an abnormality of DA mediated regulatory mechanisms. Prolactin responses to DA infusion do not differ among schizophrenics, affective patients, and controls. These findings have been verified with increased numbers of patients.

b. An integrated neuroendocrine procedure has been administered using arginine, a central releaser of growth hormone and prolactin; the hypothalamic peptide TRH, which releases TSH and prolactin; and the hypothalamic peptide LH-RH, which releases LH and FSH. The purpose of the timed infusion of these three trophic substances is to provide an evaluation of overall HP function. Among drug free depressed patients, unipolar subjects show significantly greater growth hormone and prolactin responses to arginine infusion than do bipolar subjects or controls. These data are compatible with a formulation of augmented hypothalamic serotonin function in the unipolar group, consistent with other data previously reported by this unit. In drug free bipolar patients studied longitudinally during depression and mania, the LH response to LH-RH is significantly greater in the manic phase compared with the depressed phase, suggesting possible increased endogenous LH-RH function in manic compared with depressed patients. In experimental animals, LH-RH has been shown to heighten libido; it is well known that augmented libido is a cardinal sign of mania.

Studies of basal levels of various pituitary and/or adrenal hormones drawn during this integrated neuroendocrine procedure show that depressed patients are hypercortisolemic and also show a trend toward decreased prolactin levels. The plasma levels of dihydroepiandrosterone sulphate (DHS) were found to be decreased in bipolar depressed women compared with control subjects and to be disproportionally lower compared with a simultaneously drawn cortisol level. Prolactin has been reported to stimulate dihydroepiandrosterone secretion, but it is unknown whether the basal prolactin and DHS levels in depressed patients bear any relationship to one another.

c. Tests of the HPA in affective illness and schizophrenia have focused on urinary free cortisol excretion with and without dexamethazone suppression. In collaboration with Dr. D. van Kammen and G. DeFraites we have shown that chronically psychotic drug free schizophrenic patients demonstrate significantly higher urinary free cortisol (UFC) excretion than normals. These levels are also higher than those seen in non-psychotic unipolar and bipolar depressed patients with affective illness.

Bipolar depressed patients show a significantly higher UFC than bipolar patients in the manic phase. In collaboration with Robert Post and James Ballenger, we have studied UFC excretion in rapidly cycling drug free manic and depressed patients; the UFC (taken as a mean of three values during the drug free state), cycled with changes in mood; thus, UFC is high in the depressed phase and low in the manic phase. In some patients there is premonitory decrease in UFC excretion prior to the switch into mania and premonitory increase in UFC excretion prior to the switch into depression.

As we have previously reported, UFC showed a significant positive correlation with CSF 5-hydroxyindole acetic acid accumulation after probenecid among unipolar patients. This finding is compatible with data in experimental animals which suggest that serotonin is excitatory to HPA function. To follow up on this finding we initiated a trial of the serotonin receptor blocker cyproheptadine in depressed patients with hypercortisolemia. Administration of cyproheptadine to one depressed patient produced an antidepressant response and a marked drop in cortisol excretion. Within several days, however, the patient began rapidly cycling between hypomania and depression. The hypomanias were associated with low and the depressions with high cortisol production. A more detailed look at these data revealed that premonitory shifts in UFC occurred prior to the actual mood shifts in this patient, similar to the premonitory shifts in some drug free rapid cyclers.

d. A blunted growth hormone response to amphetamine administration was reported in patients with endogenous depression. It has been suggested that the mechanism for this blunted response is due to diminished catecholaminergic function. In collaboration with Dr. Elliot Gershon and Dr. John Nurnberger of the BPB, we are studying the reproducibility and heritability of neuroendocrine responses to amphetamine in a population of normal homozygous and heterozygous twins. Both the prolactin and growth hormone responses appear to be heritable, based on the data from monozygotic twins; all subjects show reproducibility on a test-re-test paradigm. In addition, the growth hormone responses to amphetamine correlate both with basal plasma MHPG levels; and with behavioral activation following amphetamine administration.

3. Assessment of psychoactive drug effects on HP function:

The effect of common psychoative agents such as lithium, imipramine, and MAO inhibitors, as well as experimental drugs such as piribedil, carbamazepine, pimozide, zimelidine, and methadone are being assessed using neuroendocrine challenge paradigms including dopamine infusion, the integrated neuroendocrine test, apomorphine stimulation, and hypertonic saline infusion. Piribedil, a dopamine receptor agonist, significantly blunts the prolactin response to a peripheral prolactin stimulus, acting strictly at a hypothalamic locus (arginine infusion). These findings suggest that either the direct effects of piribedil or the compensatory changes in dopaminergic and/or serotonergic regulation differ at the central and peripheral receptor sites.

Both acute and chronic desmethylimipramine (DMI) administration significantly augment prolactin levels in depressed patients. These findings are compatible with recent data that show that DMI functions both acutely and chronically as a serotonin receptor agonist. The significance of these findings in terms of possible effects of DMI on endocrine dependent neoplasia, such as breast cancer, that in some instances may be partially prolactin dependent, remains to be elucidated. Acute desmethylimipramine causes significant increase in growth hormone levels in recovered but not depressed patients with primary affective disorder. This finding suggests altered alpha-adrenergic and/or serotonergic receptor function in depressed subjects. The effects of both acute and chronic desmethylimipramine and zimelidine on the basal levels of LH, FSH, and cortisol are also being assessed.

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Extein, I., Pickar, D., and Gold, P.W.: Clinical and neuroendocrine studies of opiate administration in patients with affective illness. *Psychopharmacology Bulletin*, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00449-06 CP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Outpatient Followup Studies of Manic-Depressive Patients and Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
OTHER:	Frederick Goodwin	Chief	CP/NIMH
	William Thompson	Clinical Associate	CP/NIMH
	David Gardner	Psychiatric Resident	Georgetown U.
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Gay Grover	Clinical Nurse Expert	CC Nursing
	Leon Cytryn	Medical Officer (Research)	BP/NIMH
	Martine Lamour	Guest Worker	BP/NIMH
	Marion Yarrow	Chief	LDP/NIMH
	Carolyne Waxler	Research Psychologist	LCP/NIMH
	Elliot Gershon	Chief, Unit on Psychogenetics	BP/NIMH
	Eleanor Dibble	Social Worker	BP/NIMH

COOPERATING UNITS (if any) Unit on Psychogenetics, BP/NIMH; Clinical Center Nursing; Extramural Clinical Research Branch, NIMH; McMaster University, Hamilton, Ontario; Parent Child, Inc., Wash., D.C.; UCLA, Los Angeles, California; LDP/NIMH; Univ. of Michigan, Ann Arbor, Mich.; Georgetown Univ., Washington, D.C.

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The outpatient facility is a treatment and research resource for a variety of studies related to manic-depressive illness such as: (a) the dynamics of couples group therapy as an adjunct to lithium maintenance, (b) attitudes toward and perceptions of bipolar illness in the affected families, (c) pregnancy and the post-partum period in patients and spouses, (d) child development and affective response in infants with a bipolar parent, and (e) biological and pharmacological studies of rapid-cycling bipolar patients. Specific new findings include: data on correlates of compliance with maintenance therapy; evidence that patients and spouses share many dysfunctional attitudes; evidence that many male bipolar patients are also prone to pregnancy-related or post-partum episodes; findings suggesting that 15-month old infants with a bipolar parent show unusual responses to separation in a controlled testing paradigm; and evidence that rapid-cycling bipolar patients on lithium have a strikingly high incidence of hypothalamic-pituitary-thyroid axis abnormalities.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Morris Parloff	Chief, Psychotherapy & Behavioral Interventional Center	CR/NIMH
Mariana Webster	Nurse Specialist	Parent Child
Gabrielle Carlson	Acting Director, Child Psychiatry Training Program	Neuropsych. Inst., UCLA
Athanasios Zis	Assoc. Prof. Psychiatry	U. of Michigan
Paul Grof	Prof. of Psychiatry	McMaster Univ.

Project Description:

An expansion of the Outpatient Clinic continues under the direction of its Chief, Dr. Rex Cowdry. Beginning in July, 1980, the Clinic will be staffed by two full-time psychiatrists, a half-time social worker, and a nurse expert enabling a broader range of studies in bipolar illness. We will also begin a training program which will include a half-time psychiatric resident and two social work students.

Followup studies involve 60-70 outpatients who are seen in a variety of treatment modalities: a weekly and a monthly couples' group, a weekly psychotherapy group, three monthly lithium maintenance groups, and a series of special project groups (such as the study of pregnancy in bipolar patients and the subsequent study of their infants). Limited individual therapy is available. Crisis intervention approaches are used.

The availability of long-term follow-up data for large numbers of patients with affective disorder is of critical importance to our understanding of the basic illness process and therefore, to the interpretation of the increasing number of biochemical, genetic, and pharmacotherapeutic findings. For the clinician, the choice of whether or not to place a given patient on prophylactic lithium maintenance involves at least an implicit estimate of what the natural course of the illness would be in the absence of the drug. Knowledge of the natural course of the illness is essential if the clinician is to make appropriate decisions concerning long-term prophylaxis. Our collaboration with Dr. Paul Grof focusing mainly on an analysis of the course of unipolar and bipolar illness in 334 patients studied without maintenance treatment, has continued this past year. A summary article of the findings has appeared in Psychopharmacology Bulletin.

It is known that lithium maintenance is not always effective in preventing recurrences of bipolar illness, a finding due undoubtedly to a variety of illness related factors, as well as psychosocial issues: stresses and/or non-compliance. Non-compliance in taking medication prescribed for prophylaxis of recurrent affective illness is often associated with the recurrence of symptoms, social disruption, and rehospitalization. We administered Sackett's Standardized Compliance Questionnaire to 48 outpatients in the NIMH clinics in order to identify variable that influence compliance. Findings indicated that patients with higher elevated mood scores were significantly less compliant with both medication and appointment keeping. Patient perception of good continuity of care was positively associated with appointment keeping. Patients who were married were significantly more compliant with lithium therapy; other demographic variables such as age, sex, and educational background were not of predictive value.

Having published several articles on the general benefits of combining group therapy with lithium maintenance (increased medication compliance, decreased relapse rates, and increased affective episodes), we have turned our attention to specific events and therapeutic interventions in group therapy which may contribute specifically to its efficacy with this group of patients. While data collection is in its early stages, it is already apparent that the process of studying group process may in itself be therapeutic. Asking patients to report, via questionnaire, what they saw as the significant events in each group meeting has seemingly resulted in an increased attention to group events and an increase in the patients' use of their "observing egos" to place group events in perspective.

The couples' groups also provide a major and rich source for the study of the relationship of the illness in individual family members to overall family pathology. The weekly couples' group focuses on couples in whom the husband has "dysphoric" manias, and provides an opportunity to explore the phenomenology of that particular presentation of the manic syndrome, and the distinctive dynamics that may develop both between the patient and spouse and within the couples' group. The monthly couples' group allows us to study multi-generational bipolar illness.

Most studies of the marital or family adjustment of manic-depressive patients have focused on disturbances and conflicts, that resulted from the patients' mood, behavior, or cognitive distortions. Using the Weisman Dysfunctional Attitudes Scale (DAS), which is designed to measure functional and dysfunctional cognitive perceptions prevalent in depressed patients, we tested the responses of 32 euthymic manic depressive patients and their spouses. The results showed no significant differences in the responses of patients and spouses. These findings suggest that the patient's perfectionistic standards and demands, overwhelming dependency needs, and maladaptive cognitions may be shared by the spouse. This has important implications for understanding and treating patterns of conflict in the marital relationship of the bipolar patient. We plan to extend the DAS study to other severely disturbed populations.

Another group followed in the clinic as part of a joint collaborative study with the Psychogenetics Unit consisted of six bipolar couples who were expectant parents. Three of the female index patients were withdrawn from lithium carbonate maintenance in the clinic when they indicated a wish to become pregnant. Although it was our original hope to maintain the highly vulnerable patients off lithium through lactation, two of them developed post-partum mania resulting in the hospitalization of one. An article has now been published describing this experience, the various psychodynamic features and previous treatment history of the three patients, and offering recommendations for the treatment of bipolar patients considering parenthood.

The six male infants born to these couples are participating with their parents in a study of the development of infants at risk for bipolar illness. These children are matched with controls, both of whose parents are free of any psychopathology. Each infant is studied on three occasions up to the age of 20 months. The studies include measurement of development, temperament and affect, a neurological examination, average evoked response testing a study of parental attitudes and behaviors, and a detailed family history with an emphasis on family psychodynamics. An analysis of video tapes of an infant stress paradigm is done by investigators at the University of Colorado. Preliminary analysis of these videotapes suggests that the offspring of a manic-depressive parent show a response to separation which is qualitatively similar to that shown by abused children. The significance and generalization of this finding is quite uncertain, since the index parents tended to have rather severe and frequent episodes of illness, usually including an episode in the child's first year of life. Further study of the effects of illness episodes on children are warranted. With the increased staffing available this coming year, the clinic plans to conduct a series of groups with adolescent offspring of bipolar patients to explore these issues more extensively in an older population.

Our study of post-partum psychoses in bipolar females led us to examine fatherhood as a precipitant of affective illness in bipolar males. In reviewing the history of all married bipolar fathers admitted consecutively to our ward during a nine year period (n = 40), we found that twenty-one had an episode of mania or depression during their wife's pregnancy or during the year following delivery. Puerperal psychosis among men is not a widespread phenomenon, so our finding of slightly more than 50% incidence suggests that pregnancy and childbirth may contribute to the development of psychosis in a genetically predisposed group of males. In addition, our preliminary findings suggest that there may be a subgroup of these male biplar patients who are at particular risk for post-partum psychoses and who may be identifiable by an earlier age of onset of affective illness, the presence of a history of childhood bereavement, and possibly by ordinal position in family of origin.

The first of a series of psychobiologic studies of rapid cycling manic-depressive patients has been completed. Using data from outpatients and inpatients, a significantly higher incidence of overt hypothyroidism was found in rapid cycling patients maintained on lithium (37%) than in non-rapid cycling patients on lithium (5%). If one includes more subtle abnormalities of the hypothalamic-pituitary-thyroid (HPT) axis (indicated by elevated TSH levels), abnormalities were found in 94% of rapid cyclers on lithium, compared with 35% of non-rapid cyclers. Thus, a significant association between functioning of the HPT axis and cyclicity is evident, an association which may have treatment implications.

Finally, a series of case reports are in preparation, dealing with a variety of issues: bipolar pseudodementia and lithium in the elderly; thyroid hormone potentiation of antidepressant response to phenelzine, thyroid hormone and the lithium "greys", and a rare complication of manic psychosis; extensive subcutaneous emphysema resulting from hyperventilation and Valsalva maneuvers.

Publications:

Zis, A.P., Grof, P., Webster, M., and Goodwin, F.K.: Prediction of relapse in recurrent affective disorder. Psychopharm. Bull. 16: 47-49, 1980

Targum, S.D., Davenport, Y.B., and Webster, M.V.: Post-partum mania in bipolar manic-depressive patients withdrawn from lithium carbonate. J. Nerv. Mental Dis. 167: 572-574, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01852-02 CP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Brief Outpatient Studies of Depressive Syndromes and Character Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Ed Silberman	Clinical Associate	LPP/NIMH
	Herbert Weingartner	Research Psychologist	LPP/NIMH
	Monte Buchsbaum	Chief, Section on Clin. Psychopharm	BP/NIMH
	Thomas Wise	Chairman, Dept. of Psychiatry	Fairfax Hosp.
	David Goldstein	Asst. Prof. of Psychiatry	Georgetown U.

COOPERATING UNITS (if any)
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since the great majority of patients with depression-spectrum illnesses are successfully treated outside the hospital, an outpatient facility for clinical and biological evaluation and treatment is essential, both to help assure appropriate clinical care and to develop an adequate and representative sample of affectively-ill patients for study. The clinic is developing a collaborative/consultative model, whereby community-based practitioners can refer patients for evaluation and yet continue to treat the patients themselves. A pilot project in the evaluation and treatment of unipolar depression has been conducted this past year. Studies of the borderline syndromes (neurophysiology and pharmacotherapy), including collaborations with the Departments of Psychiatry, Yale University and Georgetown University, are under way. A series of psychopharmacologic studies, including the effects of monoamine oxidase inhibitors on circadian rhythms and of vasopressin analogs on ECT-induced amnesia, are planned.

PHS-6040
(Rev. 10-76)

Project Description:

A significant re-evaluation of the Outpatient Unit has occurred over the past year, stimulated by two factors. First, patients with classical endogenous depressive symptoms are increasingly treated in the community as outpatients or on short-stay general hospital units, resulting in the selective referral of treatment-unresponsive patients and rapid cyclers to our Inpatient Unit. Second, patients with "atypical" depressions, character disorders, alcoholism, and sleep disorders are increasingly reported to have biological components to their illness. These patients also are usually evaluated and treated in outpatient settings or brief hospitalization units.

We are therefore developing a variety of new evaluation and treatment programs, including: collaborations with general hospital inpatient units, collaborations with private outpatient psychotherapists, and programs where we can provide direct clinical care. This approach reserves our inpatient resources for the longitudinal study of cyclic and treatment-resistant patients.

This year marks the completion of a pilot project in the Outpatient Clinic, CPB, that explores the feasibility of collaborative/consultative research and treatment programs involving outpatients referred by private practice psychiatrists in the Washington, D.C. area. The initial project provided for a five-day outpatient evaluation of depressed patients referred by several area psychiatrists, followed by a double-blind "drug-of-choice" treatment phase, if agreed to by the treating physician, the patient, and the clinic staff. Several conclusions can be drawn from our experience with the first 25 patients. First, the patients are a more representative sample of the spectrum of depressed patients than those referred to NIH for inpatient evaluation and treatment. Second, there are major advantages to the collaborative model, since private psychiatrists can continue to provide psychotherapy and continuity of care (thus benefiting the patient), and since this model encourages the development of an ongoing relationship between the referring psychiatrist and the clinic staff. The expansion of staffing of the Outpatient Clinic occurring in July 1980 will allow a full-scale series of studies in depression-spectrum illness, involving differential diagnosis, studies of circadian rhythms, and studies of neuroendocrine and neurotransmitter abnormalities.

The availability of additional staff will also enable the clinic to embark on a series of outpatient studies of other disorders. A study of the borderline syndrome, reported by us this year at the Annual Meeting of the American Psychiatric Association, suggests that individuals diagnosed as borderline have symptomatology suggestive of limbic system dysfunction and have a high incidence of abnormal electroencephalograms. A controlled study of neurophysiological and neurochemical abnormalities in this syndrome is under way. Collaborations with the Yale and Georgetown Departments of Psychiatry will explore the efficacy of specific drug regimens in the inpatient treatment of borderline patients. Collaborations with psychiatrists in the Washington, D.C. area will explore outpatient psychopharmacologic treatment of this syndrome.

Finally, the availability of a new version of our solid-state self-contained activity monitor - one which continuously quantifies levels of activity over 8-day periods during outpatient evaluation and treatment - will enable a series of studies of response to psychopharmacologic agents, including a longitudinal outpatient study of the effects of monoamine oxidase inhibitors on circadian activity and sleep patterns.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01831-04 CP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Basic and Clinical Studies of the Neuronal and Glial Enolases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As reviewed in last year's annual report, a new and distinct form of the glycolytic enzyme enolase has been isolated and characterized from both rat and human brain. Immunohistochemical studies have established that this protein is strictly localized in neurons and neuroendocrine cells and it has been named Neuron Specific Enolase or NSE. An additional enolase isoenzyme has also been isolated and shown to be localized in glial cells and designated Non-Neuronal Enolase or NNE. These isoenzymes represent the first example of cell specific isoenzyme markers for neurons and glia. Recent efforts have focused on the development of the central nervous system, where it has been established that NSE only appears in differentiated neurons. Neuronal differentiation involves an NNE to NSE switch; this phenomenon is being exploited in a wide variety of developmental neurobiological studies. NSE is also a specific marker for neuroendocrine peptide secreting cells of the APUD class; immunohistochemical studies are in progress in which NSE is being used to map the diffuse neuroendocrine nervous system. Using a sensitive radioimmunoassay, clinical studies of NSE and NNE levels in cerebrospinal fluid and amniotic fluid are being correlated with neuronal and glial pathologies.

PHS-6040
(Rev. 10-76)

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M. Del Cerro	Professor, Ctr. for Brain Res.	Rochester
M. Whitehead	Research Associate	U. of Conn.
R. Herndon	Chairman, Ctr. for Brain Res.	Rochester
Donald Schmechel	Neurology Resident	Duke University
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Cooperating Units (if any) continued:

Hopkins University Medical Center; National Naval Medical Center; UMEA, Sweden; Mayo Clinic, Rochester, Minnesota; Duke University; University of London

Project Description:

Much of our current understanding of central nervous system (CNS) function is derived from the germinal efforts of pioneer investigators such as Ramon y Cajal and Golgi who painstakingly visualized the complex cytoarchitecture of the brain using histochemical techniques. These studies originated the concepts of individual neurons, synaptic junctions and cell to cell communication. As valuable as the Golgi staining method has proven to be, it suffers from the shortcoming that only about 1% of neurons stain and the mechanism of staining remains unknown. Recent studies have therefore focused on functional markers for neurons and glia so that the myriad cell to cell relationships in nervous tissue might be better visualized.

Since the brain is a complex system of highly differentiated cells, one approach to specific identification of neurons has involved an attempt to identify proteins which exist only in brain. These brain proteins might in turn be involved in the specific differentiated functions of brain neurons. Previously reported studies conducted in our laboratory have shown that a specific neuron contains isoenzyme of the glycolytic enzyme enolase designated neuron specific enolase (NSE). A glial specific form of the enzyme has also been described, which we have designated non-neuronal enolase (NNE). Structural studies have shown that NSE is a dimer composed of two apparently identical γ subunits (dimer M.W. 78,000 Daltons). NNE is also a dimer composed of two α subunits (dimer M.W. 87,000 Daltons). NNE and NSE have been established to be the product of separate genes and have been shown to differ markedly in their functional properties. NSE is much more stable than NNE towards inactivation by chloride, a property which suits NSE to its specific neuronal localization where chloride levels can be quite high. Antisera to NSE does not react with NNE and vice versa. The availability of specific antisera for a neuronal and a glial protein conveys the ability to both visualize (immunocytochemistry) and quantitate (radioimmunoassay) these two basic components of the central nervous system (CNS).

Our research efforts that concern neuronal and glial enolases have focused on two general areas. The first centers on the issue of what specific metabolic demands exist in brain, or more specifically in the neuron, that require the existence of a structurally, immunologically, and functionally distinct form of enolase? The fact that NSE only appears in differentiated neurons suggests that this unique enolase is required for the specific energy demands of differentiated neuronal functions (i.e., impulse conduction, neurotransmitter synthesis, etc.). The second major research focus encompasses a much broader range of studies and involves the use of NNE and NSE as probes of CNS morphology, pathology, and development and as markers for the diffuse neuroendocrine nervous system.

During the past year some basic findings have been reported that concern the physiology of NSE. A phylogenetic study has shown unequivocally that NSE is only present in vertebrate and bird nervous tissue, and not in lower species. This is an intriguing finding since it suggests that higher nervous systems are not only more sophisticated morphologically but also biochemically. This finding also suggests that the metabolic demand that necessitates the existence of NSE is unique to vertebrate nervous tissue.

Studies that concern the amount of NSE in various subcellular fractions of brain tissue and purified neuronal and glial cell preparations are done in collaboration with Dr. Solomon Snyder in Baltimore. These studies were initiated last year, using the ^{3}[H] RIA procedure, they are continued using the more sensitive ^{125}[I] RIA. The results of these studies may redefine our previous conceptions of "synaptosomal" fractions, since these fractions contain NNE as well as NSE. Different methods of synaptosomal preparation are analyzed for NNE and NSE in an effort to determine the optimal procedure for obtaining pure neuronal components. It may well be that many older studies performed with synaptosomal fractions should be repeated with fractions whose purity has been established by their NSE and NNE content.

During the past year considerable effort has been directed towards the study of neuronal differentiation using the recently demonstrated "switch" from NNE to NSE as an index of neuronal maturation. Since our initial demonstration of this unique phenomenon, in both rat and monkey brain, we have shown that the switch also seems to occur in primary cultured neurons. This has been demonstrated in two different cell culture systems, in collaboration with Dr. Cara Schengrund at Hershey Medical School (dissociated primary neuronal cultures) and Drs. Trapp and Webster at the NIH (brain aggregate cultures). In both systems the NSE/NNE ratio increases in a coincident fashion with neuronal morphological maturation, as demonstrated by both immunocytochemical staining and radioimmunoassay. In both culture systems the level of NSE attained remained about 5-fold below that observed in vivo suggesting that these cells remained only partially differentiated. Extensions of these studies are planned in which the effects of electrical and chemical stimulation will be evaluated with respect to their effect on NSE levels and NSE/NNE ratios. Controlled studies of development and aging in primary neuronal cultures offer an attractive means to study the life cycle of these cells. Immunocytochemical staining of cultured cells for NSE has proven to be a very valuable aid in their identification and characterization. We have provided serum to approximately 20 research groups over the past year for this purpose.

Neuronal maturation is also being studied in vivo in collaboration with other research groups, including Drs. Manuel Del Cerro and Ludwig Sternberger at the University of Rochester, where the developmental profile is being examined in the retina. The well-defined cell types in retina and their known functional interrelationships make this tissue attractive as a system for the correlation of specific neuronal functions with the NNE-NSE switch. Preliminary results in this study have shown impressive staining profiles in mature tissue. It has also been shown that glutaraldehyde fixed tissue can be successfully stained for NSE, allowing examination of standard pathology preparations. This basic observation permits the analysis of many pre-existing samples for NSE content. This procedure is being prepared for publication as a method paper.

In collaboration with Dr. Hanno Koppel of London, the development of the external granule cell layer is being studied in rat cerebellum. These studies are aimed at elucidating the precise timing of the NNE to NSE switch and attempting to correlate it with known neuronal events. Similar studies are also in progress in collaboration with Drs. Mark Whitehead and Ken Morest at the University of Connecticut in the chick auditory system. Preliminary results from this study indicate that NSE appears at a time coincident with synaptogenesis in this system.

The recent demonstration of NSE in peripheral neuroendocrine cells of the APUD series is of great interest since these cells have been considered to be paraneuronal in nature. The presence of NSE in these cells, shown by both immunocytochemistry and radioimmunoassay, has provided perhaps the best evidence for this hypothesis to date. In collaboration with Drs. Julia Polak and A.G.E. Pearse, we are currently involved in characterizing the APUD cell series. Results obtained to date indicate that gut cells that contain putative neuropeptides (such as gastrin and somatostatin) also contain NSE. These results suggest that new putative neuropeptide secreting cells might be easily identified by NSE staining, a strategy that is currently being pursued. Application of the NSE methodology to the APUD system offers a convenient means of mapping the entire diffuse neuroendocrine nervous system. Successful completion of such a task will undoubtedly provide new insights that concern the interrelationship of the CNS with peripheral organ systems.

NSE has recently been shown to be present in blood platelets. These studies, done in collaboration with Drs. Campbell (London) and Murphy (NIMH), are consistent with other studies showing that platelets have a number of functions analogous to neurons.

Studies of mesodermally and ectodermally derived muscle cultures are being done in collaboration with Dr. Vanda Lennon at the Mayo Clinic in Minnesota. These studies have shown that ectodermally derived muscle cells undergo a transitory phase in development where they contain NSE. Mesodermally derived muscle does not display this transitory neuron-like phase. The results derive from both immunocytochemical and radioimmunological data. The results of this intriguing study are currently being prepared for publication; they suggest that muscle cells derived from ectoderm undergo a "neuron-like" phase that distinguishes them from other muscle cells. The transitory expression of NSE in muscle cells derived from the neural ectoderm represents the first tangible biochemical similarity between neurons and muscle cells, the two major excitable cells in the vertebrate organism.

Motivated by the assumption that the cancer cell represents a reversal of differentiation, the process of neuronal and neuroendocrine cell transformation is also of interest. In collaboration with Dr. Harland Coons of the National Cancer Institute, neuronal transformation by a temperature sensitive virus is being studied. By adjusting the temperature of the growth media, cells can become transformed; NSE levels are being correlated with this process in the hope of demonstrating transformation dependent changes that might be attributed to changes in the state of neuronal differentiation. Preliminary results have been very encouraging since cells grown at the permissive temperature (33° C) contain about 10-fold less NSE than the same cells grown at the restricted temperature (40° C) where the cells are not transformed.

This system provides a powerful model for the study of the biochemistry of neuronal transformation. Rat PC-12 pheochromocytoma cells are also being studied in collaboration with Dr. Gordon Guroff at the NIH. Since nerve growth factor (NGF) causes profound morphological alterations in these cells, the effect of NGF on NSE levels will be assessed in this system as well.

The high levels of both NSE and NNE in brain, coupled with their strict respective neuronal and glial localization, predict their usefulness as potential diagnostic probes of neuronal and glial pathologies. Elevated levels of NSE in cerebrospinal fluid (CSF) may be a consequence of neuropathy and high levels of NNE a consequence of gliopathy. In order to test this hypothesis a more sensitive RIA procedure was developed using ^{125}[I] labeled NSE. This assay is 50-fold more sensitive than the ^{3}[H] RIA and we were able to determine accurate normal CSF NSE levels. Using this assay, our study of human amniotic fluid NSE levels in neural tube defect pregnancies has been completed this past year. Very significant increases in NSE levels are correlated with both spina bifida and anencephalic fetuses. In addition, the two abnormalities can be distinguished from each other, since NSE levels are significantly higher in anencephalic material compared with spina bifida. This represents the first demonstration of elevated cell specific protein levels being correlated with a developmental abnormality and suggests that NSE may prove useful in the specific diagnosis of neural tube disorders.

A study of NSE levels in schizophrenic CSF, done in collaboration with Drs. Weinberger, Potkin, and Wyatt at St. Elizabeth's Hospital, has produced negative results: no significant elevation in NSE was observed. Studies are in progress with neurological patient material supplied by the St. Elizabeth's group and from Dr. Robert Herndon at the University of Rochester. Conditions to be studied include Huntington's chorea, Altzheimer's disease, and head trauma.

The demand for NSE, NNE, and their antisera has grown considerably during the last year with an increased number of neurobiologists requesting our reagents for neuroanatomical, developmental, and pathological studies. Negotiations are still in progress with various commercial suppliers for the marketing of these reagents. Several companies have expressed interest and we anxiously await the availability of these reagents so as to relieve us from our current role as the supplier of these materials. Commercial availability of NSE and NNE RIA kits and serum for immunocytochemical staining should establish this methodology as the method of choice for both quantitative and qualitative studies of the CNS and diffuse neuroendocrine nervous systems where information that relate to cellular identity or state of differentiation is desired. It should also stimulate further biochemical and physiologic studies that concern the physiologic role of this unique enolase isoenzyme and shed light on the basic metabolic demands specific to neurons and neuroendocrine cells.

Publications:

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Clark-Rosenberg, R.L. and Marangos, P.J.: Phylogenetic distribution of neuron specific enolase. J. Neurochem., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01834-03 CP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Endogenous Ligands for the Brain Benzodiazepine Receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Sanford Markey	Chief, Section on Pharm. Applic. of Mass Spec.	LCS/NIMH
	Bruce Trapp	Staff Fellow	LNNS/NINCDS
	Jacqueline Crawley	Staff Fellow	CP/NIMH

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1.9	0.6	1.3

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The recent demonstration of pharmacologically relevant receptor sites for the benzodiazepines in brain provide insight into the molecular mechanism of action of these drugs. The presence of receptor sites for benzodiazepines predicts the existence of naturally occurring ligands. Studies from our laboratory have shown that the purines specifically inhibit ^{3}[H] diazepam binding to brain receptor sites, that they possess "benzodiazepine-like" pharmacologic and neurophysiologic properties, and that structure-activity relationships exist. Inosine has been shown to antagonize seizures produced by caffeine, suggesting that the benzodiazepine receptors and their putative endogenous ligands may be involved in seizure modification. Several higher molecular weight benzodiazepine receptor ligands have also been isolated, although they have not yet been identified. Recent studies have centered on the interaction of the GABA and benzodiazepine receptors: both the purines and benzodiazepines have been shown to be potent inhibitors of ^{3}[H] diazepam binding, as are the methylxanthine stimulants caffeine and theophylline. Furthermore, these compounds have been shown to be an order of magnitude more potent as inhibitors of GABA-stimulated diazepam binding compared with basal binding.

Project Description

The existence of apparently functional receptor sites for neurotransmitters, neuromodulators and drugs in the central nervous system (CNS) offer a very simple means of studying some of the characteristics of these systems. Since the receptor is the recognition site for a particular neuroactive substance, it represents only the first and probably the simplest step in what is probably a series of biochemical events that produce a cellular response. Simple receptor binding assays also offer an extremely valuable tool for screening substances (i.e., drugs) that might have pharmacologic properties mediated by a specific neurotransmitter system.

The benzodiazepines represent a fascinating group of substances due to their very marked and quantifiable anxiolytic, anticonvulsant, and muscle relaxant effects. It is conceivable that anxiety, seizure activity, and centrally mediated muscle tone represent a spectrum of phenomena mediated by one or more neuronal systems within the CNS. The early literature on benzodiazepine action described multiple effects of these drugs on the cholinergic and monoaminergic neurotransmitter systems but raised considerable doubt as to whether these effects were directly mediating benzodiazepine action or were merely epiphenomena. The recent discovery of a stereospecific, saturable receptor site for the benzodiazepines in the CNS indicates that these drugs are affecting a distinct, heretofore undescribed, neuromodulator system and not simply affecting previously known systems. This finding further suggests that a naturally occurring endogenous ligand for the benzodiazepine receptor should exist, much like the discovery of the opiate receptor predicted the existence of the endorphins. The isolation and characterization of endogenous ligands for the benzodiazepine receptor might therefore provide very basic insights into the homeostatic mediation of anxiety, much as the endorphins have expanded our conceptions of analgesics.

By extracting large quantities of brain tissue, fractionating these extracts on various chromatographic systems, and assaying the fractions for their ability to inhibit specific diazepam binding to synaptosomal membranes, endogenous inhibitors of binding were found. Employing an organic extraction procedure several active factors were identified which after extensive analysis proved to be the purine riboside inosine and the purine base hypoxanthine. These two compounds are low potency inhibitors of diazepam binding with observed K_I values of 750 μM. Extensive analysis of all other known naturally occurring purines revealed distinct structure-activity relationships with the 2-deoxy-purine derivatives being in all cases more potent than their parent compounds (Ki = 250 μM). Methylation of the purine nucleus in various positions also had pronounced effects on inhibitory potency. Extensive testing of purines in other receptor systems (i.e., opiate, cholinergic, dopaminergic) revealed no effects, indicating that they are highly specific in their interaction with the benzodiazepine receptor. The purines are also without effect on the kidney benzodiazepine binding site, indicating that this site differs from the brain specific receptor.

Since the methylxanthine stimulants caffeine and theophylline are methylated purines they were tested and found to be very potent inhibitors of diazepam binding. The respective potencies of these compounds as inhibitors of binding directly parallels their potencies as cortical stimulants, a finding that supports the intriguing hypothesis that some of the pharmacologic effects of these ubiquitous, widely consumed stimulants, may be mediated by the benzodiazepine receptor. To test this hypothesis further a study has been completed during the past year in our laboratory concerning caffeine-induced seizures. High doses of caffeine have been reported to cause tonic-clonic seizures. Since this phenomenon had never been systematically studied we undertook a study aimed at the characterization of these seizures and their modulation by the benzodiazepines and purines. Doses of caffeine in the range of 200-250 mg/kg (in mice) produce classic "pentylenetetrazol-like" seizures. These seizures are antagonized by the benzodiazepines, with a rank order potency that directly parallels their potency as inhibitors of diazepam binding, that suggests that the receptor is mediating this phenomenon and that caffeine and benzodiazepine represent an agonist-antagonist pair with respect to seizure generation. Furthermore, the active purine inosine partially antagonizes caffeine-induced seizures whereas the inactive purine 7-methyl-inosine is without effect. The purines therefore are "benzodiazepine-like" in this system, and caffeine has the reverse effect. These studies involving caffeine-induced seizures have been submitted for publication. Recent neurophysiological studies performed by Polc et al. at Roche-Basel have supported this interpretation. In these studies the increase in spinal cord dorsal root potentials and dorsal root reflexes induced by diazepam is specifically blocked by both caffeine and theophylline without affecting other spinal cord activities. It therefore seems that future studies that concern the mechanism of action of caffeine might well focus on the interaction of this substance with the benzodiazepine receptor.

As part of our effort to characterize the purines, their behavioral effects have been studied in collaboration with Dr. Jacqueline Crawley, a PRAT Fellow who has recently joined our Branch. In these studies it has been shown that low doses of inosine, but not 7-methyl-inosine, inhibit the increase in both locomotor and exploratory activity caused by diazepam administration. Since higher doses of diazepam cause sedation rather than increased activity, it is not yet clear whether inosine is inhibiting or potentiating the diazepam effect. These results do however provide further support, at the behavioral level, for the hypothesis that the purines are modulating the benzodiazepine receptor system.

A relatively broad base of data indicates that purines are neuroactive compounds and that one of their sites of action is the benzodiazepine receptor. These compounds competitively inhibit diazepam binding, display "benzodiazepine-like" neurophysiologic and pharmacologic properties and are present in the central nervous system. The physiologic relevance of the purines is still clouded by the lack of information concerning their metabolism and localization in brain. The multiplicity of active purines is also problematic and presents problems similar to those encountered with the endorphins, where a growing list of active peptides has given rise to difficulties in ascertaining the functions of each. Regional distribution studies of individual purine levels may help resolve some of these questions.

Collaborative studies were started during the past year with Dr. Eberhard Trams at NINCDS concerning the distribution of purines and purine ribosides in brain. A high pressure liquid chromatograph (HPLC) assay for the purines has been developed that makes it possible to quantitate the levels of a spectrum of purines simultaneously. Recent studies are aimed at measuring the levels of various purines with known activity at the benzodiazepine receptor and determining whether in vivo quantities sufficient to have effects on the receptor exist. Since rapid postmortem metabolism of purines is a major methodologic problem, microwaved animals are used. Preliminary results have revealed high levels of inosine and hypoxanthine (µMolar) using a 2 second microwave beam. These will be compared with more extensively denatured brain tissue (4 and 6 second beam) to insure that no postmortem metabolic interconversion has occurred. The results of these studies are of paramount importance since it is critical that purine levels sufficient to affect the benzodiazepine receptor be demonstrated, if one is to ascribe physiologic relevance to these substances.

It has been well documented that the benzodiazepine and GABA receptor systems are functionally coupled. The benzodiazepines enhance GABA-mediated neurophysiological effects, and GABA stimulates ^{3}[H] diazepam binding in vivo. Prompted by reports that the convulsant benzodiazepine RO-3663 inhibits GABA-stimulated diazepam binding while having little effect on basal diazepam binding in the absence of GABA, we have extensively studied GABA-stimulated diazepam binding and its inhibition by both benzodiazepines and purines. This very recently concluded study has shown that benzodiazepines, purines and nicotinamide all inhibit GABA-stimulated benzodiazepine binding with much higher potency than basal binding. The IC_{50} for inosine inhibition of GABA-stimulated binding is 130 µM and for 2-deoxyinosine and 2-deoxy-guanosine is 30-40 µM. In general the purines were about an order or magnitude more potent as inhibitors of GABA-stimulated diazepam binding than were the benzodiazepines. The lower levels of purines necessary to inhibit stimulated binding also greatly increases the likelihood that physiologically relevant levels of these compounds exist in brain. The studies relative to the inhibition of GABA-stimulated diazepam binding have been submitted for publication.

The relative inhibitory potencies of the benzodiazepines differ, depending on whether one assesses basal binding or GABA-stimulated binding. Diazepam is 20-fold more potent than chlordiazepoxide as an inhibitor of GABA-stimulated binding whereas it is 60-fold more potent in basal binding inhibition. The closer relative potencies of diazepam and chlordiazepoxide for inhibition of stimulated binding is more consistent with their clinical potencies (i.e., chlordiazepoxide doses are about 4-5 times lower than diazepam doses) suggesting that inhibition of GABA-stimulated diazepam binding may be more relevant to the action of these drugs than is basal binding. The markedly different potencies of benzodiazepine receptor ligands as inhibitors of basal compared with GABA-stimulated diazepam binding indicates that GABA either induces a change in the diazepam receptor or that two populations of benzodiazepine receptors exist in brain. Studies aimed at differentiating between these two possibilities are currently in progress. Preliminary results show a lower degree of GABA-stimulated binding in cerebellum relative to cortex and suggest that two populations of receptors exist. Further study of each binding profile under different physical conditions should prove sufficient to resolve this question. It is possible that multiple states of the benzodiazepine receptor exist in brain with a restricted population coupled to the GABA receptor-chloride ionophore complex.

Prompted by the apparent close functional coupling of the benzodiazepine and GABA receptors, we have recently developed the capacity within our own laboratory of assaying both low and high affinity GABA receptor sites. We have used a new 4, ^{3}[H] Muscimol derivative and devised conditions where 80% specific binding is obtained. Two binding sites are observed with apparent K_D values of 20 and 200 µM. We are currently completing a study that involves chronic benzodiazepine administration; both the GABA and benzodiazepine receptor are being analyzed. Marked subsensitivity is induced in the benzodiazepine receptor after a three week treatment course with clonazepam (30-50% decrease in B max) a finding reproduced in three separate experiments. The decreased B max persists for 6-10 days after drug discontinuation, after which time the binding profile returns to normal. Preliminary analysis of these animals reveals that in both cortex and cerebellum ^{3}[H] Muscimol binding is increased. Chronic clonazepam administration therefore seems to have opposite effects on the GABA binding site. We are currently investigating the effect of lithium (administered in the diet) on the regulation and interaction of these receptor systems. The work describing the induced subsensitization of this benzodiazepine receptor has been submitted for publication. The Muscimol binding experiments are in the process of being repeated prior to submission for publication.

In addition to studies that characterize the purines as putative benzodiazepine receptor ligands we have also, during the last 18 months, isolated from bovine and rat brain two additional higher molecular weight fractions that competitively inhibit diazepam binding. These factors have been designated Peak I and Peak II and have estimated molecular weights of 5,000-10,000 daltons and 500-600 daltons, respectively. Both factors also inhibit GABA-stimulated diazepam binding with approximately 10-fold greater potency than basal binding. Progress in identifying these two new factors has been painstaking but some of their physiochemical properties have been determined. Both factors are heat stable and stable to degradation by proteolytic and nuclease enzymes. They are not soluble in organic solvents of varying polarity such as ether and butanol. Initial attempts by ion exchange chromatography to further purify Peak I and II proved unfruitful. Recently, preparative HPLC has been used and Peak I has been resolved into two fractions. Future experiments using polyacrylamide gel electrophoresis are planned and should yield highly purified material. Reverse phase thin layer chromatography (TLC) and HPLC of Peak II has also yielded a more purified preparation. Identification of these further purified preparations is being planned in collaboration with Dr. Sanford Markey of LCS using mass fragmentography and gas chromatography. Pharmacologic, neurophysiologic and behavioral studies of the purified factors will determine their biological relevance.

An understanding of the physiologic role of the benzodiazepine receptor is dependent on the characterization of the endogenous ligand for the receptor. Although a growing list of ligand candidates are emerging in the literature, the purines remain by far the best candidates for this role. Further characterization of this system and its relationship to GABA-ergic inhibitory systems in brain may provide germinal insights into the neurochemistry of anxiety and convulsant activity in the CNS.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01836-02 CP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of CNS Neurotransmitter and Drug Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Moshe Rehavi	Visiting Fellow	CP/NIMH
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	Jeffrey Barker	Medical Officer	LNP/NINCDS
	John MacDonald	Visiting Scientist	LNP/NINCDS
	Martin Zata	Medical Officer	LCS/NIMH
	Kenner Rice	Pharmacologist	LC/NIAMDD
	Evan Williams	Visiting Fellow	LBC/NIAMDA
	Kwan-Ling Lock	Guest Worker	LBC/NIAMDD
	Ryotaro Tsujimura	Guest Worker	LBC/NIAMDD

COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIAMDD; Laboratory of Neurophysiology, NINCDS; Laboratory of Chemistry, NIAMDD; Laboratory of Clinical Science, NIMH.

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.5	PROFESSIONAL: 2.2	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Saturable, high affinity and stereospecific binding sites for benzodiazepines have been demonstrated both in vivo and in vitro in the mammalian central nervous system. The correlations between the binding of a series of benzodiazepines in vitro and their pharmacologic potencies as anxiolytics, anticonvulsants, and muscle relaxants strongly suggests these sites are receptors mediating the therapeutic actions of the benzodiazepines. Recent evidence suggests these receptors may be functionally linked to receptors for GABA (γ-aminobutyric acid) and a chloride-ionophore. This macromolecular complex may mediate benzodiazepine-sensitive states such as anxiety, muscular tension, and seizure activity. Saturable and high affinity binding sites for imipramine have been demonstrated in membrane preparations from human platelet and human brain. These binding sites are apparently related to the serotonin reuptake site and are distinct from the cholinergic, α-adrenergic, and histaminergic receptors. The peripheral (platelet) and central binding sites appear to have similar, if not identical, binding characteristics. There is a significant decrease in the density of these imipramine binding sites in platelets from depressed patients when compared to platelets from age- and sex-matched healthy volunteers.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To characterize and determine the role and regulation of the benzodiazepine-GABA-chloride ionophore "receptor" complex. To isolate, identify and evaluate endogenous substances isolated from mammalian brain which modulate this macromolecular complex. To develop suitable model systems for *in vitro* study of this complex. To develop novel compounds useful in studying benzodiazepine receptor function. To develop potentially useful therapeutic agents using information obtained from identification of endogenous ligands and modulators of this complex. To understand the pharmacological and physiological significance of the "high affinity" binding sites for [^{3}H] imipramine in brain and platelet. To characterize and purify the presynaptic reuptake (i.e., transport) site for serotonin in platelets and brain.

Methods Employed: Neurochemical characterization of the benzodiazepine-GABA-chloride ionophore receptor-complex have been studied using radioreceptor assays employing radiolabelled benzodiazepine and GABA (as well as GABA analogs). Isolation and identification of endogenous modulators of the benzodiazepine-GABA-chloride ionophore receptor-complex has been accomplished with column chromatography, high pressure liquid chromatography, and differential filtration techniques. Pharmacologic therapy of compounds has largely been assessed by the anticonvulsant activity of various compounds along with measurement of spontaneous motor activity. Genetic animal models such as the spontaneous hypertensive and the pink-eyed, tan-hooded rat have been used to assess the physiologic role of this benzodiazepine-GABA-chloride ionophore receptor-complex. Radioligand binding assays using [^{3}H] imipramine were employed. Potential irreversible inhibitors (i.e., covalently bound blockers) are being synthesized in order to further characterize these binding sites and to establish their exact relationship to the serotonin reuptake site.

Major Findings: Primary cultures of fetal mouse brain and spinal cord possess benzodiazepine receptors which are pharmacologically similar to those found in the mammalian brain. These primary cultures may thus be a valid model to study the function of the benzodiazepine-GABA-chloride ionophore receptor-complex *in vitro*.

Mammalian retina contains both "brain-type" and "kidney (peripheral)-type" benzodiazepine receptors. These two types may be clearly resolved with the use of appropriate ligands with selectivity towards brain or peripheral-type receptor (e.g., RO-5-4864). Retina may thus prove a useful model to study the interrelationship of these two types of binding sites. Studies using intravitreal injection of kainic acid, neonatal administration of monosodium glutamate, and pink-eyed, tan-hooded rats suggest "brain-type" receptors are localized to neuronal elements of the rat retina (e.g., inner plexiform layer) and are not on other neuronal elements such as photoreceptor cells.

Heterogenous populations of benzodiazepine receptors in brain were revealed using a novel alkylating agent, kenazepine. These populations of receptors have a differential chemical reactivity to this agent, which most likely reside at or near the active center of the receptor. Marked regional differences in the proportions of these receptor subtypes are observed. In vivo administration of kenazepine and another alkylating benzodiazepine (irazepine) produces a long lasting protection against pentylenetetrazole-induced convulsions when compared with equimolar administration of "classical" (non-alkylating) benzodiazepine such as diazepam.

Studies designed to explore the minimal structural requirements necessary for purines to bind to the benzodiazepine receptor were initiated. These studies were prompted by our previous finding suggesting that the purines inosine and hypoxanthine are endogenous ligands of the benzodiazepine receptor. Adenosine, a structurally related purine with a very low affinity for the receptor (greater than 2000 μM), was chosen as a starting material. Increasing the lipophilicity of adenosine by addition of halogenated heterocycles at the N^{-6} position results in an almost 100 fold increase in potency. A prototype compound (KM-12) was evaluated in this laboratory and demonstrated to be a potent anticonvulsant. Further evaluation is being conducted by the ADD program of the NINCDS. Preliminary results appear encouraging, and this compound is now being evaluated in pre-clinical trials for long term toxicity.

Introduction of a bridged compound in the N^{-6} position of adenosine (e.g., bicycloheptyl or bicyclooctyl) yields compounds which increase (rather than competitively inhibit) [^{3}H] diazepam-binding in vitro. Kinetic analysis revealed this increase in [^{3}H] diazepam binding is due to an increase in site number rather than affinity. This is the first example of benzodiazepine receptor number being increased in vitro as a result of drug treatment. These increases are stereospecifically antagonized by the GABA-antagonist, bicuculline, yet are independent of GABA concentrations in the medium. These compounds do not affect [^{3}H]GABA or [^{3}H]muscimol (a GABA-agonist) binding in vitro. These data suggest that such compounds (e.g., KM-13) may increase site number in vitro by displacement of an endogenous noncompetitive inhibitor of [^{3}H] diazepam binding normally present near the benzodiazepine-GABA-chloride ionophore receptor-complex. These data further suggest that a bicuculline-sensitive site plays a permissive role in this effect, i.e., the bicuculline site must be in an "open" (unblocked) conformation for these effects to occur. In vivo administration of KM-13 to mice results in an increase in receptor number as well. This compound appears to possess long-lasting anticonvulsant, anxiolytic, and sedative properties, reminiscent of the benzodiazepines. This compound is being evaluated by the ADD program of the NINCDS.

Further, low molecular weight compound(s) have been isolated from rat brain which competitively inhibit [^{3}H] diazepam binding. These compounds are

7-20 fold more potent in inhibiting GABA-enhanced benzodiazepine binding compared with basal (non-GABA enhanced) binding. The data suggest that the GABA-benzodiazepine coupling mechanism may be an important locus for both the action of endogenous factors and pharmacologic agents.

Levels of diazepam present in the brain at the time of a profound electrophysiological effect (potentiation of adenosine-elicited depression of firing of cortical neurons) are nearly two orders of magnitude less than those needed to block adenosine uptake in vitro. These data, taken together with previous findings from our laboratory, strongly suggest that the pharmacologic effects of benzodiazepines are not related to a blockade of adenosine uptake in situ, and hence that the central effects of benzodiazepines are not dependent on activation of adenosine receptors.

High affinity and saturable binding sites for [^{3}H] imipramine have been demonstrated in membrane preparations from human brain and platelet. These binding sites appear to be specific for tricyclic antidepressants since specifically bound [^{3}H] imipramine is displaced by pharmacologically-active tricyclic antidepressants at low concentrations. Tertiary amine antidepressants such as imipramine and amitriptyline are more potent inhibitors of [^{3}H] imipramine binding than are the corresponding secondary amines nortriptyline and desipramine, suggesting a relationship between the high affinity binding sites and the serotonin reuptake site. In contrast, other psychotropic drugs and neurotransmitter agonists and antagonist have little or no effect on specific [^{3}H] imipramine binding. The characteristics of [^{3}H] imipramine binding in human brain membranes are very similar (if not identical) to those of human platelets, suggesting that the latter may be a useful model in studying the pharmacological significance of these binding sites. Preliminary studies of [^{3}H] imipramine binding in platelets from severely depressed patients reveal a significant ($\geq$ 30%) decrease in binding site density (B_{max}) when compared to platelets from healthy age-matched controls.

Significance to Biomedical Research and the Program of the Institute: The benzodiazepines and tricyclic antidepressants are among the most widely prescribed drugs in current therapeutic use. Understanding their mechanism of action and identification of endogenous compounds which may function as "benzodiazepine-like" or "antidepressant-like" compounds is of fundamental importance to the understanding of diseases such as epilepsy, anxiety-neuroses, alcoholism, and depression, as well as development of a more fundamental understanding of brain function. In addition, these studies may provide information regarding the development of therapeutic agents superior to those currently available.

Proprosed Course of Project: The significance of the benzodiazepine-GABA-chloride ionophore receptor-complex relative to the pharmacologic actions of anxiolytics other than the benzodiazepines (e.g., propanediol carbamates) and anticonvulsants other than benzodiazepines (e.g., barbiturates) will be investigated. The search for endogenous modulators of the benzodiazepine-GABA-chloride ionophore receptor-complex will be extended to include factors which may be displaced by purines such as KM-13. Attempts at receptor purification will be made using the novel benzodiazepines developed in this laboratory. The hypothesis that many convulsant/anticonvulsant agents act through modulation of the GABA-benzodiazepine-chloride ionophore receptor-complex will be further examined.

Further studies on [^{3}H] imipramine binding to platelets from patients with a variety of psychiatric disorders including depression will be conducted. Purification and characterization of the high affinity binding site for [^{3}H] imipramine from platelet and brain will also be carried out.

Publications:

MacDonald, J.F., Barker, J.L., Paul, S.M., Marangos, P.J., and Skolnick, P.: Inosine may be an endogenous ligand for benzodiazepine receptors in cultured spinal neurons. Science 205: 715-717, 1979.

Paul, S.M., Syapin, P.J., Paugh, B.A., Moncada, V. and Skolnick, P.: CNS benzodiazepine receptors: Correlation between receptor occupation and anticonvulsant effects of diazepam. Nature 281: 688-689, 1979.

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Skolnick, P., Paul, S.M., and Marangos, P.J.: Brain benzodiazepine levels following intravenous administration of [^{3}H] diazepam: Relationship to the potentiation of purinergic depression of CNS neurons. Canad. J. Physiol. Pharmacol. 57: 1040-1042, 1979.

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Huang, A., Barker, J.L., Paul, S.M., Moncada, V.Y., and Skolnick, P.: Characterization of benzodiazepine receptors in primary cultures of fetal mouse brain and spinal cord. Brain Res. 190: 485-492, 1980.

Paul, S.M., Zatz, M., and Skolnick, P.: Demonstration of "brain-specific" benzodiazepine receptors in rat retina. Brain Res. 187: 243-246, 1980.

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Skolnick, P., Lock, K.-L., Paugh, B.A., Moncada, V., Marangos, P., Windsor, V., and Paul, S.M.: Pharmacologic effects of EMD 28422, a novel purine which enhances [^{3}H] diazepam binding. Pharmacol. Biochem. Behav., in press.

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Paul, S.M., Marangos, P., Brownstein, M., and Skolnick, P.: Demonstration and characterization of an endogenous inhibitor of GABA-enhanced [^3H] diazepam binding from bovine cerebral cortex. In Costa, E., di Chiari, G., and Gessa, G.L. (Eds.): GABA and Benzodiazepine Receptors. New York, Raven Press, in press.

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Paul, S.M., Rehavi, M., Skolnick, P., and Goodwin, F.K.: Demonstration of "high affinity" binding sites for [^3H] imipramine on human platelets. Life Sci. 26: 953-959, 1980.

Paul, S.M. and Crews, F.T.: Rapid desensitization of cerebral cortical β-adrenergic receptors induced by desmethylimipramine and phenoxybenzamine. Eur. J. Pharmacol. 62: 349-350, 1980.

Skolnick, P., Paul, S.M., Zatz, M., and Eskay, R.: "Brain-specific" benzodiazepine receptors are localized to the inner plexiform layer of rat retina. Eur. J. Pharmacol., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01838-01 CP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Studies on the Interaction(s) of Peripheral Hormones with CNS Neurotransmitter Receptors.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Sally Hays	Research Associate	CP/NIMH
	Andrew Hoffman	Research Associate	LCS/NIMH
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	Margery Beinfeld	Guest Worker	LCS/NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.7	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Significant behavioral and neuroendocrine effects of a variety of peripheral hormones including the gonadal steroids, as well as the pituitary and gastrointestinal peptides have been described by a number of investigators. Little data exists as to the neurochemical mechanisms involved in the interaction of these hormones with excitable tissues; including central neurotransmitter systems. Studies in our laboratory have been designed to elucidate the possible biochemical effects of hormones on neuronal preparations in vivo and in vitro. Significant effects of biologically active estrogens on catecholamine efflux or "release" from the isolated hypothalamus in vitro have been demonstrated. The conversion of estrogens to novel catechol metabolites (catechol estrogens) by the brain and other neuroendocrine tissues has also been reported. Recent studies on the gastrointestinal peptide cholecystokinin have been undertaken and the presence of specific receptors for CCK have been demonstrated in the CNS.

Project Description:

Objectives: To study the neurochemical effects of various peripheral hormones on neurotransmitter-related events, including transmitter release, turnover, and receptor sensitivity. To identify specific recognition or receptor sites for peripheral hormones within the CNS. To study the conversion of hormones by target tissues to more active or less active metabolites.

Methods Used: Radioreceptor assays, organ and tissue culture, radioenzymatic assays for neurotransmitters and catechol estrogens, radioimmunoassay of cyclic nucleotides and hormones.

Major Findings: Incubation of hypothalamic explants with biologically active estrogens resulted in a significant increase in norepinephrine efflux during a 70 min. incubation. The time course and dose-response characteristics of the estrogen efflux of norepinephrine were similar to effects of estrogen on cyclic AMP accumulation in the same tissue preparation. The synthesis of catechol estrogens by brain and other neuroendocrine tissues was studied in the rat. The liver followed by brain had the highest enzyme activity and nearly all tissues possessed some estrogen-2-hydroxylase activity. Significant differences in enzyme activity were noted between male and female rats and the effects of cytochrome P450 enzyme inducers and inhibitors were studied.

The binding of biologically active ^{125}I cholecystokinin to rat brain membranes was established. This binding was of high affinity, saturable, reversible and inhibited by biologically-active cholecystokinin peptides. Kinetic and competition studies suggest that these high affinity binding sites represent physiologically relevant receptors for CCK in brain.

Significance to Biomedical Research: An understanding of the neurochemical mediators of hormone action within the CNS will have broad implications to a number of disease states in man. Additionally, since a number of hormones have been reported to have behavioral and mood effects, and since abnormal endocrine homeostasis may be involved in the pathophysiology of affective illness, the precise neurochemical events mediating hormone action may be helpful in elucidating neurochemical substrates in depression.

Proposed Course of Project: The present studies will be expanded to examine the effects of various endocrine manipulation(s) and drugs on catechol estrogen synthesis in brain and liver. In addition, the possible neurophysiologic effects of catechol estrogens will be examined in cultured pituitary and neuronal preparations. The effects of age, diet, and psychotropic drugs on CCK receptors in various regions of the brain will also be examined.

Publications:

Paul, S.M., Axelrod, J., Saavedra, J., and Skolnick, P.: Estrogen-induced efflux of endogenous catecholamines from the hypothalamus in vitro. Brain Res. 178: 499-505, 1979.

Paul, S.M. and Axelrod, J.: Catechol estrogens: Localization, formation, and actions in brain and other estrogen-sensitive tissues. In DiCarlo, F. Genazzani, E., and Mainwaring W.I.P. (Eds.): Pharmacological Modulation of Steroid Action. New York; Raven Press, 1980, pp. 1-10.

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Hoffman, A.R., Paul, S.M., and Axelrod, J.: Estrogen-2-hydroxylase in the rat - Distribution and response to hormonal manipulation. Biochemical Pharmacology 29: 83-87, 1980.

Hoffman, A.R., Paul, S.M., and Axelrod, J.: The enzymatic formation of catecholestrogens from 2-methoxyestrogens by rat liver microsomes. Endocrinology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00422-09 LCS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Neuropharmacology of Circadian Rhythms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Zatz, Medical Officer (Research)	LCS NIMH
Others:	M. Herkenham	LNP NIMH
	D. Jimerson	APB NIMH

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Laboratory of Neurophysiology, DBBR, NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

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NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Circadian rhythms and environmental lighting regulate a number of endocrine and behavioral functions. A paradigm has been established for the evaluation of drug effects on the mechanisms generating circadian rhythms. This has been applied to the regulation of activity rhythms in mice. The chick pineal provides an in vitro model of a vertebrate circadian oscillator. The role of cyclic nucleotides in the regulation of circadian rhythms is being investigated.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To elucidate the neuropharmacology of circadian rhythms.

Methods Employed: Biochemical, chromatographic, chemical, pharmacologic, behavioral, radioactive trace techniques.

Major Findings: Intraventricular administration of carbachol (a cholinergic agonist) is capable of phase-shifting the activity rhythms of free-running mice. Both phase-delays and phase-advances were observed. These effects are themselves phase-dependent. These observations confirm and extend observations made on the central regulation of the circadian rhythm in the rat pineal gland. They provide the first demonstration of a neurotransmitter's effect on the circadian oscillator.

Chick pineals show a circadian rhythm in indoleamine metabolism similar to that of rats. However, unlike rats, their rhythm is not driven by adrenergic stimulation. Treatment with cholera toxin, which stimulates adenylate cyclase, can reveal an otherwise cryptic role for cyclic nucleotides.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, temperature, and other physiologic functions. Elucidation of the mechanisms generating or regulating circadian rhythms are of broad clinical and biologic interest.

Proposed Course of Project: Mechanisms generating and regulating circadian rhythms and the effects of light will be explored further.

Publications:

Zatz, M. and O'Dea, R.F.: Synthesis and release of cyclic GMP by nerve endings in the rat pineal gland and neurohypophysis. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.): Catecholamines: Basic Clinical Frontiers. Proceedings of the Fourth International Catecholamine Symposium. New York, Pergamon Press, 1979, pp. 247-249.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00425-04 LCS

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Peripheral and Central Catecholamines in Hypertension and Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Saavedra, Medical Officer (Psychiatry)	LCS	NIMH
Others:	T. Torda, Visiting Fellow	LCS	NIMH
	F. Correa, Guest Worker	LCS	NIMH
	R. del Carmine, Guest Worker	LCS	NIMH
	G. Feuerstein, Visiting Fellow	LCS	NIMH
	A. Negro-Vilar, Assoc. Prof., Southwest University, Texas		
	N. Alexander, Assoc. Prof., University Southern California		
	E. Krieger, Prof., University Sao Paulo, Brazil		
	P. Setler, Smith, Kline & French Laboratories		

COOPERATING UNITS (if any)

Southwest University, Texas; University of California; University of Sao Paulo, Brazil; Smith, Kline & French Laboratories

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20206

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the role of catecholamines in the central and peripheral nervous system in experimental and genetic hypertension and stress and has now extended to study the participation of other neurotransmitters (amines and neuropeptides) in the central regulation of a number of autonomic functions.

Central catecholamines play a role in all forms of hypertension, genetic and experimental, studied, and in stress. Blockade of synthesis of adrenaline coexists with decreased blood pressure in hypertensive rats. Inhibitors of adrenaline synthesis could have antihypertensive and antianxiety effects. Alterations of peripheral β-receptors and in endogenous opiates exist in genetic hypertensive rats. Changes in the metabolism of membrane phospholipids are present in genetically hypertensive rats.

Central amines (catecholamines, serotonin, histamine) are involved in the regulation of sodium intake and in the formation of hypothalamic vasopressin.

Project Description:

Objectives: To study the role of central and peripheral catecholamines in hypertension and stress and to study the interaction between brain monoamines and neuropeptides in the regulation of autonomic functions.

Methods Employed: Anatomical: microdissection of brain nuclei. Surgical: lesions of brain nuclei by specific neurotoxins. Lesion of brain by knife cuts. Implantation of chronic cannulas in brain nuclei and lateral ventricles. Surgical baroreceptor deafferentation. Biochemical: enzymatic isotopic micromethods for the measurement of biogenic amines and related enzymes. Radioimmunoassays for neuropeptides; immunofluorescent techniques to localize brain peptides.

Major Findings: Neurogenically hypertensive (sino-aortic denervated) rats present increased plasma catecholamine levels three days after operation. These results suggest an early activation of the peripheral adrenergic system after supression of the afferent nerves from the baroreceptors to the brain, and can explain the tachicardia present in this early stage of hypertension.

Sodium dependent, hypertensive rats (Dahl rats) show changes in peripheral (heart and pineal) catecholamines. These results suggest that peripheral catecholamines may play a role in the development of the sodium-dependent hypertension.

Spontaneously hypertensive rats (SHR) present increased stimulation of pineal β-receptors over normotensive controls. This increased β-receptor activation coexists with increased methylation of pineal membrane phospholipids, indicating that the activation of membrane-bound phospholipid methyltransferases can play a role in hypertension. SHR also present localized changes in hypothalamic histamine levels. These results could explain the action of exogenously administered histamine on blood pressure, and help to clarify the mechanism of action of centrally-acting antihypertensive drugs such as clonidine.

Genetic hypertensive rats show a markedly increased latency in their reflex responses to pain which is naloxone-dependent. These results indicate a modification of the endogenous opiate metabolism or of the opiate receptors in spontaneously hypertensive rats.

Brain catecholamines present progressive changes during the development of Doca-salt hypertension in rats. Thus, changes in central amines can also occur as a consequence of increased blood pressure.

During stress, the adrenaline-forming enzyme, PNMT, is decreased in forebrain areas. There appear to be important differences between forebrain and brain stem methyltransferases, suggesting that these enzymes are different or that they are regulated by different physiological mechanisms.

Rats homozygous for diabetes insipidus, deficient in vasopressin (Brattleboro rats), show specific changes in catecholamines, serotonin and histamine, which correspond to the vasopressin-forming areas of the hypothalamus and are partially corrected by parenteral administration of vasopressin. These results suggest a role for catecholamines, serotonin and histamine in control of vasopressin regulation and sodium metabolism.

The enzymatic isotopic method for histamine has been modified with a 5-fold increase in sensitivity.

Proposed Course of Project: A combination of drug treatments, endocrine additions, central lesions and other neuroendocrine manipulations will be performed in different neuroendocrine models. Special interest will be focused on: 1) the role of central adrenergic neurons in stress, hypertension and anxiety; 2) the role of serotonin and histamine in hypertension, stress and neuroendocrine regulation; 3) the interactions between neuropeptides and biogenic amines in the central regulation of autonomic functions; 4) the nature of the changes in β-adrenergic receptors and phospholipid methylation in cell membranes in hypertension; 5) the possible use of adrenaline-synthesis inhibitors and other drugs in anxiety states; 6) the role of endogenous opiates in hypertensive states; 7) the application of sensitive and specific enzymatic-isotopic assays for biogenic amines and metabolites to human studies in depression and anxiety.

Significance to Biomedical Research: Inhibitors of adrenaline synthesis are now being developed for application to studies on anxiety in human subjects. The efficacy of inhibitors of adrenaline synthesis to decrease high blood pressure could result in the use of these or similar drugs for the treatment of some forms of hypertension in humans. The studies on central neurotransmitters could help to explain some aspects of the regulation of autonomic functions by the brain. The continuing development of new microtechniques for the measurement of biogenic amines and related compounds allows their determination in small clinical samples and in microgram amounts of brain tissue and are used in clinical investigations and in the study of the role of discrete areas of the brain.

Publications:

Saavedra, J.M.: Brain catecholamines during development of DOCA-salt hypertension in rats. Brain Res. 179: 121-127, 1979.

Saavedra, J.M.: Microquantitation of neurotransmitters in specific areas of the central nervous system. International Review of Neurobiology 21: 259-274, 1979.

Saavedra, J.M., del Carmine, R., Iwai, J. and Alexander, N.: Catecholamines in discrete areas of the rat brain in different forms of genetic and experimental hypertension. In Albertinia, A., Da Prado, M. and Peskar, B.A. (Eds.), Radioimmunoassay of Drugs and Hormones in Cardiovascular Medicine. New York, Elsevier North Holland Biomedical Press, 1979, pp. 199-215.

Young, R.A., Robinson, D.S., Vagenakis, A.G., Saavedra, J.M., Lovenberg, W., Krupp, P.P. and Danforth, E., Jr.: Brain TRH, monoamines, tyrosine hydroxylase and tryptophan hydroxylase in the woodchuck, Marmota monax, during the hibernation season. Comp. Biochem. Physiol., 63c: 319-323, 1979.

Saavedra, J.M.: Central adrenergic neurons in spontaneous (genetic) and experimental hypertension. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.), Catecholamines: Basic and Clinical Frontiers, Vol. II. Neuroregulator Interactions, Lesions and Histochemistry, Clinical Aspects. New York, Pergamon Press, 1979, pp. 1416-1418.

Paul, S.M., Axelrod, J., Saavedra, J.M. and Skolnik, P.: Estrogen-induced efflux of endogenous catecholamines from the hypothalamus *in vitro*. Brain Res. 178: 499-505, 1979.

Grobecker, H., Saavedra, J.M., McCarty, R., Weise, V.K. and Kopin, I.J.: Role of noradrenergic nerves and adrenal medulla during the development of genetic and experimental hypertension in rats. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.), Catecholamines: Basic and Clinical Frontiers, Vol. I. Catecholamine Metabolism, Receptors, Neuropharmacology. New York, Pergamon Press, 1979, pp. 906-908.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00426-04 LCS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Catecholestrogens: Synthesis and Developmental Aspects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Hoffman, Research Associate	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
Others:	M. Poth, Clinical Associate	IRP NICHHD
	S. Paul, Chief, Unit on Preclinical Pharm.	CPB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Catecholestrogens are important metabolites of estrogen which have significant biological properties of their own. They are synthesized by 2-hydroxylation of estrogen in brain and in liver and by demethylation of 2-methoxyestrogens in the liver. Both brain and liver of estrogen-2-hydroxylase show sexual dimorphism, and the enzyme can be measured in the fetus in both organs.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the developmental aspects of estrogen metabolism and to learn how this development is regulated.

Methods Used: Enzymatic, endocrinologic, pharmacologic.

Major Findings: In liver, estrogen-2-hydroxylase could be detected after 17 days' gestation. The specific activity of the enzyme increased logarithmically until one week of age and then linearly until early puberty. Then, the level in the female liver remained constant and the activity in the male liver rose sharply to a level 7 times that of the female.

In brain, the enzyme could be detected at 19 days' gestation. In late puberty, the levels in the male increased to 5 times that of the female. This sexual differentiation occurred several weeks later than the differentiation in liver.

The enzyme in the female liver could be induced by one injection of testosterone given after day 14, but the brain enzyme could not be induced.

Since male alcoholics become feminized, we studied the liver enzyme in rats made dependent upon ethanol. Ethanol caused a rapid decrease in liver enzyme activity and this diminished activity persisted for one week.

Significance to Biomedical Research: Catecholestrogens are important metabolites of endogenous and exogenous estrogens. The ability to metabolize estrogens changes with age and may be dependent upon prior hormone exposure. The differential ability to metabolize estrogens may play a role in the development of estrogen-sensitive neoplasms (e.g., carcinoma of the breast).

Proposed Course of Project: The effect of intrauterine exposure to drugs (phenobarbitol), and hormones on the ability of the adult animals to metabolize estrogens will be studied.

Publications:

Hoffman, A.R., Paul, S.M. and Axelrod, J.: Catecholestrogen synthesis and metabolism by human breast tumors *in vitro*. Cancer Res. 39: 4584-4587, 1979.

Hoffman, A.R., Paul, S.M., and Axelrod, J.: The enzymatic formation of catecholestrogens from 2-methoxyestrogens by rat liver microsomes. Endocrinology (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00427-03 LCS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Phospholipid Methylation and Signal Transduction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Hirata, Visiting Scientist	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
	F. Crews, Research Associate	LCS NIMH
	D. Bareis, Research Associate	LCS NIMH
	A. Hotchkiss, Research Associate	LCS NIMH
	R. Del Carmine, Guest Worker	LCS NIMH
Others:	T. Hoffman, Senior Scientist	LID NCI
	V. Hubbard, Clinical Associate	PM NIAMDD
	J. Tallman, Research Biologist	BP NIMH
	M. Hatanaka, Research Biologist	LVC NCI
	Y. Itoh, Visiting Scientist	NCI
	M. Nirenberg, Chief, Laboratory of Biochemical Genetics	BG NHLBI
	J. Daly, Chief, Laboratory of Biorganic Chemistry	BC NIAMDD

(see attached)

COOPERATING UNITS (if any) NCI: LID, LVC; NIAMDD: PM, BC, D, CH: NHLBI: BC, CM: NIDR: LDBA, LMI. Department of Immunology, JHU. Department of Biochemistry, NYSU

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 5.0	PROFESSIONAL: 5.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Stimulation of IgE receptors on rat mast cells and basophilic leukemia cells causes a transient increase of phospholipid methylation followed by disappearance of methylated lipids due to the activation of phospholipase A_2. In rat mast cells, blocking phospholipase A_2 results in the inhibition of Ca influx as well as in the inhibition of arachidonic acid release. The close relation between phospholipid methylation and $Ca2^+$ influx is found in rabbit peritoneal leucocytes, human fibroblast, and mouse lymphocytes. Phospholipase A_2 is regulated by its specific inhibitory protein induced by corticoid in rabbit neutrophils and human fibroblast. The phospholipase A_2 inhibitory protein is being isolated and characterized.

PHS-6040
(Rev. 10-76)

Names, Laboratory and Institute Affiliations and Titles of Principal Investigators and All Other Professional Personnel Engaged on the Project

Others (cont):	L. Harrison, Visiting Scientist	D	NIAMDD
	R. Siraganian, Chief, Section on Clin. Immun.	LMI	NIDR
	M. Vaughan, Chief, Laboratory of Cellular Metabolism	CM	MHLBI
	K. Ishizaka, Professor of Immunology, Johns Hopkins University		
	C. Ventor, Assistant Professor of Biochemistry, New York State University		
	E. Schiffman, Research Chemist	LDBA	NIDR
	R. Schulman, Chief, Clinical Hematology Branch	CH	NIAMDD
	R. Henneberry, Research Biologist	LMB	NINCDS

Project Description:

Objectives: To study the role of phospholipid methylation in a mechanism of signal transduction across the membrane.

Methods Employed: Enzymic, radiometric, immunological, pharmacological.

Major Findings: Work done in the past year has shown that phospholipid methylation and subsequent activation of phospholipase A_2 are involved in the signal transduction by various receptors. Previously we have already demonstrated that the stimulation of β-adrenergic receptors in rat reticulocytes, Hela cells and C_6 astrocytoma cells increases the phospholipid methylation, membrane fluidity and coupling of the β-adrenergic receptors to adenylate cyclase. Long term stimulation causes the degradation of methylated lipids and simultaneously uncouples the β-adrenergic receptors from adenylate cyclase. Stimulation and inhibition of phospholipase A_2 by activators (phorbol esters, etc.) and inhibitors (mepacrine, etc.) resulted in stimulation and inhibition of this uncoupling.

In rat mast cells or basophilic leukemia cells, stimulation of IgE receptors by anti-IgE receptor antibody or IgE plus anti-IgE or ovalbumine resulted in a transient increase of phospholipid methylation. This increase precedes Ca^{2+} influx, arachidonate and histamine release. When phospholipid methylation is inhibited, Ca^{2+} influx and arachidonate release as well as histamine release are blocked in a dose-dependent fashion. A similar association between Ca^{2+} influx and phospholipid methylation and physiological response was observed in rabbit neutrophils (chemotaxis) and human fibroblast (cyclic AMP generation).

Glucocorticoid treatment of neutrophils and fibroblast resulted in loss of their chemotaxic responses and inhibition of phospholipase A_2 was measured by arachidonate release. Further studies revealed that protein(s) with the molecular weight of 40,000 daltons was induced and that this protein can inhibit phospholipase A_2 *in vitro*.

In collaboration with Dr. Nirenberg's lab, where many monoclonal antibodies for membrane proteins have been isolated, we surveyed the presence of antibodies for β-adrenergic receptor, methyltransferase(s) and phospholipase A_2 inhibitory protein(s). Preliminary experiments suggested such candidates for each protein.

Significance to Biomedical Research: Phospholipid methylation and phospholipase A_2 appear to be involved in coupling and uncoupling of β-receptors to adenylate cyclase (super- and subsensitivity), histamine release (allergy), leucocyte chemotaxis and fibroblast response (inflammation, phagocytosis). Furthermore, phospholipid methylation appears to modulate Ca^{2+} influx, a cation involved in many biologic functions.

Proposed Course of Project: Purification and characterization of methyltransferases will be continued. Properties of antibodies for β-adrenergic receptors, methyltransferase and phospholipase A_2 inhibitor protein(s) will be characterized and their antibodies should be applied for the survey of their normal biological functions and their role in rheumatic diseases.

Publications:

Hirata, F., Axelrod, J. and Crews, F.T.: Concanavalin A stimulates phospholipid methylation and phosphatidylserine decarboxylation in rat mast cells. Proc. Natl. Acad. Sci., USA, 76: 4813-4816, 1979.

Hirata, F., Corcoran, B.A., Venkatasubramanian, K., Schiffmann, E. and Axelrod, J.: Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. Proc. Natl. Acad. Sci., USA, 76: 2640-2643, 1979.

Hirata, F., Strittmatter, W.J. and Axelrod, J.: Phospholipid methylation, membrane fluidity and coupling of the beta-adrenergic receptor. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers, Vol. 5. New York, Pergamon Press, 1979, pp. 501-504.

Paul, S.M., Axelrod, J. and Skolnick, P.: Estrogen dependent efflux of endogenous catecholamines from the hypothalamus in vitro. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.), Catecholamines: Basic and Clinical Frontiers, Vol. 2. New York, Pergamon Press, 1979, pp. 1206-1208.

Strittmatter, W.J., Gagnon, C. and Axelrod, J.: Beta-adrenergic stimulation of protein carboxylation and amylase secretion in rat parotid. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.), Catecholamines: Basic and Clinical Frontiers, Vol. 1. New York, Pergamon Press, 1979, pp. 339-401.

Strittmatter, W.J., Hirata, F., Axelrod, J., Mallorga, P., Tallman, J.F. and Henneberry, R.C.: Benzodiazepine and beta-adrenergic receptor ligands independently stimulate phospholipid methylation. Nature, 828: 857-859, 1979.

Crews, F.T., Morita, Y., Hirata, F., Axelrod, J. and Siraganian, R.P.: Phospholipid methylation affects immunoglobulin E-mediated histamine and arachidonic acid release in rat leukemic basophils. Biochem. Biophys. Res. Commun., 93: 42-49, 1980.

Hirata, F., Tallman, J.C., Jr., Henneberry, R.C., Mallorga, P., Strittmatter, W.J. and Axelrod, J.: Regulation of beta-adrenergic receptors by

phospholipid methylation. In Pepeu, G., Kuhar, M.J. and Enna, S.J. (Eds.) Advances in Biochemical Psychopharmacology, Vol. 21. Receptors for Neurotransmitters and Peptide Hormones. New York, Raven Press, 1980, pp. 91-97.

Hirata, F., Toyoshima, S., Axelrod, J. and Waxdal, M.J.: Phospholipid methylation: A biochemical signal modulating lymphocyte mitogenesis. Proc. Natl. Acad. Sci., USA, 77: 862-865, 1980.

Ishizaka, T., Hirata, F., Ishizaka, K. and Axelrod, J.: Stimulation of phospholipid methylation, Ca^{2+} influx and histamine release by bridging of IgE receptors on rat mast cells. Proc. Natl. Acad. Sci., USA, 77: 1903-1906, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00429-01 LCS

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biosynthesis of Nonpolar Methylated Lipids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Zatz, Medical Officer (Research)	LCS	NIMH
Others:	P. Dudley, Senior Staff Fellow	LVR	NEI
	S. Markey	LCS	NIMH
	S. Paul	CPB	NIMH
	C. Latker	LVR	NEI
	L. Eiden	LCS	NIMH

COOPERATING UNITS (if any)

Laboratory of Vision Research, NEI
Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several tissues incorporate methyl groups from S-adenosylmethionine into nonpolar lipids. This appears to be an enzymatic process occurring in cell membranes. Identification of these nonpolar lipids and investigation of their metabolism should lead to the elucidation of their physiologic function.

Project Description:

Objectives: To identify the nonpolar lipids formed from S-adenosylmethionine and to investigate their biochemistry.

Methods Employed: Biochemical, chromatographic, chemical, pharmacologic, behavioral, and radioactive trace techniques.

Major Findings: Incubation of membranes from lung retina, or salivary gland with radioactive S-adenosyl methionine (SAM) resulted in the formation of several radioactive lipids. The major products were nonpolar neutral lipids which migrated on one dimensional TLC like fatty acid methyl esters (FAME). Evidence suggesting that these nonpolar lipids were formed enzymatically includes: 1) Their formation was time, temperature and protein dependent; 2) Heat denatured preparations showed no activity; 3) S-adenosyl homocysteine (a competitive inhibitor of transmethylation reactions using SAM) markedly inhibited the reaction; 4) Free methanol (in concentrations 1000 times greater than SAM) did not inhibit; 5) The reaction displayed an "apparent affinity" for SAM of approximately 1 μM; 6) Activity varied considerably among tissues; 7) Activity was highest in microsomes but present in other membranous fractions, including plasma membranes. Column chromatography resolved the nonpolar lipids into at least 2 fractions, only one of which coelicited with FAME. The formation of FAME was confirmed by gas chromatography with radiodetection and by GC-mass spectrometry using isotopic ratios. Palmitic, stearic, and linoleic acid methyl esters were identified. The remaining, as yet not fully identified, lipid has been characterized by HPLC, GC, and GC-MS. Its formation predominates over that of FAME in whole cells. It is labile and appears to be capable of releasing methanol upon degradation.

The rat retina contains diazepam receptors of the "brain" type which are modulated by GABA. They are localized in the inner plexiform layer.

Significance to Biomedical Research: The role of membrane lipids in cellular function, particularly in signal transduction, is currently being recognized and elucidated. These newly discovered lipids may play a dynamic role in cellular function.

Proposed Course of Project: The structure, biochemistry and pharmacology of the newly discovered nonpolar lipids will be investigated.

Publications:

Zatz, M.: Low concentrations of lithium inhibit the synthesis of cyclic AMP and cyclic GMP in the rat pineal gland. J. Neurochem. 32: 1315-1321, 1979.

Paul, S.M., Zatz, M. and Skolnick, P.: Demonstration of "brain-specific" benzodiazepine receptors in rat retina. Brain Res. 187: 243-246, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00430-01 LCS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Tricyclic Antidepressants, Alpha-adrenergic Blocking Agents and Response of Beta-adrenergic Receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Crews, Research Associate	LCS	NIMH
Other:	S. Paul, Chief, Unit on Preclinical Pharmacology	CPB	NIMH

COOPERATING UNITS (if any)
Unit on Preclinical Pharmacology, Clinical Psychiatry Branch, NIMH

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Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The delay in the onset of action of antidepressant therapies is a major clinical problem. Studies in rats have shown that desensitization of cerebral cortical β-receptors requires several weeks of treatment with antidepressant drugs, similar to the time course required for the onset of therapeutic actions. β-receptor desensitization is thought to be essential for antidepressant action. Combined treatment with desipramine, a tricyclic antidepressant, and phenoxybenzamine, an α-adrenergic receptor blocker, cause a rapid desensitization of cerebral cortical β-receptors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine if α-receptor blockade during antidepressant treatment can rapidly desensitize β-adrenergic receptors.

Methods Employed: Radioactive, receptor binding.

Major Findings: Combined treatment with desipramine and phenoxybenzamine rapidly decreases the number of β-adrenergic binding sites in rat cerebral cortex.

Significance to Biomedical Research: This is the first *in vivo* demonstration of rapid desensitization of central nervous system β-receptors. These findings may lead to an antidepressant therapy with a rapid onset of action and thereby eliminate a major clinical problem.

Proposed Course of Project: Continued studies to determine if α-receptor blockade increases the onset of action of other antidepressant treatment, e.g., monoamine oxidase inhibition and electro-convulsive treatment.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00431-01 LCS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Lipid metabolism, Membrane Viscosity and Alcohol Dependence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Crews, Research Associate	LCS	NIMH
	F. Hirata, Visiting Scientist	LCS	NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS	NIMH
Other:	E. Majchrowitz, Research Biologist, Laboratory of Preclinical Studies		NIAAA

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chronic administration of ethanol produces dependence and tolerance. The induction of ethanol dependence in rats is associated with an alteration in the viscosity of the synaptosomal plasma membrane (SPM). There is also a marked increase in the amount of cholesterol in the SPM of animals dependent on and tolerant to ethanol. Two phospholipid methylating enzymes are localized in the SPM. The activity of these two enzymes is decreased in SPM of animals dependent on ethanol.

Project Description:

Objectives: To study the changes in membrane composition and phospholipid methylating enzymes which occur during the induction of dependence and tolerance to ethanol in rats.

Methods Employed: Fluorescent, enzymatic and radiometric.

Major Findings: The viscosity of the synaptosomal plasma membranes (SPM) from rats dependent on and tolerant to ethanol is increased. The *in vitro* addition of ethanol to SPM decreases the viscosity of membranes; however, membranes prepared from animals dependent on ethanol are resistant to this fluidizing effect of ethanol. A marked increase in the amount of cholesterol in the SPM was found in rats dependent on ethanol. The *in vitro* addition of cholesterol to control membranes caused changes in viscosity similar to the changes which occur in membranes of animals dependent on ethanol.

Two phospholipid methylating enzymes are localized in SPM. The activity of these enzymes is reduced in SPM of animals dependent on ethanol. The reduction occurs primarily in the first and rate-limiting enzyme.

Significance to Biomedical Research: Ethanol causes changes in nervous system function which could be due to alterations in membrane viscosity. A resistance to the fluidizing action of ethanol may be related to tolerance to and dependence on ethanol. Changes in membrane composition and phospholipid methyltransferases, which affect membrane viscosity, may be a common factor to the numerous changes in nervous system function caused by ethanol administration.

Proposed Course of Project: The effects of ethanol treatment on membrane composition and phospholipid methylation will be further characterized. Changes in transmitter receptor, function, e.g., β-receptor-adenylate cyclase coupling, which could be due to membrane alterations, will be examined. The mechanism of the changes in membrane composition will be studied.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00432-01 LCS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Protein Carboxyl-methylation: A post translational modifier of protein function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Hoffman, Research Associate	LCS NIMH
	Y. Kloog, Fulbright Scholar/PHS Int'l. Fellow	LCS NIMH
	A. Hotchkiss, Research Associate	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
Other:	C. Gagnon, Faculty of Medicine, Laval University;Quebec, Canada	

COOPERATING UNITS (if any)

Department of Pharmacology, Laval University; Quebec, Canada

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Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Protein carboxyl methylase (PCM) is a ubiquitous soluble enzyme which reversibly carboxylates glutamic acid residues of many acidic proteins. Calmodulin is a protein which regulates the activity of a number of Ca^{++}-requiring enzymes in brain and elsewhere, and is an excellent substrate for PCM. When calmodulin is methylated, its ability to enhance the activity of phosphodiesterase is inhibited. The acetylcholine receptor is a complex of 5 polypeptides and is also an excellent substrate for PCM. Certain acidic phospholipids will inhibit PCM activity, implying that PCM may be regulated by cell membrane constituents.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To learn which proteins are good substrates for protein carboxyl methylase (PCM) and to determine how their activity is altered by the carboxylation.

Methods Employed: Enzymatic, pharmacologic.

Major Findings: Calmodulin is a protein which regulates the activity of a number of Ca^{++}-requiring enzymes in brain and elsewhere, and is an excellent substrate for PCM. When calmodulin is methylated, its ability to enhance the activity of phosphodiesterase is inhibited.

The acetylcholine receptor is a complex of 5 polypeptides and is also an excellent substrate for PCM.

Certain acidic phospholipids will inhibit PCM activity, implying that PCM may be regulated by cell membrane constituents.

Significance to Biomedical Research: Carboxymethylation may prove to be a key regulatory step in the activity of a number of enzymes and receptors by reducing negative charges on membrane proteins.

Proposed Course of Project: The precise location of methylation of the ACh receptor will be delineated and the functional consequences of methylation will be investigated. The possibility that PCM is involved with exocytosis will be investigated by studying the effect of PCM on isolate adrenal chromaffin granules.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00401-15 LCS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Formation, Release, Disposition, and Metabolism of Neurotransmitters in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	David C. Jimerson	Senior Staff Fellow	LCS NIMH
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	Nguyen B. Thoa	Pharmacologist	LCS NIMH
	Virginia K. Weise	Chemist	LCS NIMH
	Isamu Yamaguchi	Guest Worker	LCS NIMH
	Giora Feuerstein	Visiting Associate	LCS NIMH

COOPERATING UNITS (if any)

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Section on Histopharmacology

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Medicine

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.8	1.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to elucidate the biochemical mechanisms controlling the synthesis, storage, release, action and termination of action of neurotransmitters and to determine how genetic factors, environmental stimuli, or drugs influence these control mechanisms. The autonomic responses to stress appear to be genetically determined and differ among strains of rats. There is a relationship between the degree of behavioral activation and elevation of plasma catecholamines found during stress. In addition to differences in the magnitude of response of the central nervous system, in spontaneously hypertensive rats there is a greater sensitivity of the arterioles to norepinephrine, whether released from the nerve endings or administered intravenously. The central control of sympathodrenal activity involves cholinergic mechanisms which interact with dopaminergic neurones as well. Presynaptic α_2-adrenoceptors are present on axonal sprouts. The affinity of various agonists to these receptors parallels their ability to inhibit release of ^{3}H-NE.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Stimulation of the sympathetic outflow from the spinal cord of pithed rats affords a means of evaluating, in the absence of compensatory cardiovascular reflexes, the release of norepinephrine from the sympathetic nerve endings and the pressor response elicited by the released catecholamine. Studies in pithed spontaneously hypertensive (SHR) and the normotensive Wistar-Kyoto (WKY) rats of the same strain show that there is no significant difference in the amounts of norepinephrine released when the same intensity of stimulation is applied to the sympathetic outflow, but in SHR rats, the B.P. response is greater. These observations suggested that the sensitivity of the blood pressure to norepinephrine released at sympathetic nerve endings was greater in SHR rats. This conclusion was supported by the observation that the B.P. elevation induced by administration of catecholamines was greater in SHR rats than in WKY rats. There appear to be two factors responsible for this difference. In young SHR rats the β-adrenoceptor mediated vasodilatory responses to catecholamines are less than in WKY rats so there is a net enhancement of arterial resistance to flow. In older SHR rats, however, structural changes appear to account for the greater arterial resistance. (Yamaguchi and Kopin).

Similar studies were performed with other forms of experimental hypertension. Pressor responses elicited by sympathetic nervous system stimulation in Grollman (renal) hypertensive but not in DOCA (deoxycortico-sterone)-salt treated hypertensive rats were significantly greater than the corresponding changes seen in the appropriate controls. Stimulation-elicited increases in plasma norepinephrine and epinephrine were no different from control in Grollman hypertensive rats and may have been slightly attenuated in DOCA-salt animals. Since maximal pressor responses to intravenously administered norepinephrine were significantly greater with respect to control in these forms of experimental hypertension, changes in peripheral end organ responsiveness to sympathetic stimulation may play a significant role in the development and/or maintenance of blood pressure elevation (DiMicco and Kopin).

Hemorrhage elicits reflex sympatho-adrenal medullary activation and increases plasma catecholamine levels in anesthetized rats (Dr. Feuerstein and Kopin). Prostaglandins of the E-series elevate blood pressure and heart rate, enhance the sympathetic response, and increase the survival of hemorrhaged rats. These effects appear to be mediated by a renal factor, since they are not seen in nephectomized hemorrhaged rats.

Although the final metabolic excretion products of norepinephrine are well known, the pathways and sites of formation of these products is still unclear. The appearance in plasma of dihydroxyphenylglycol (DHPG) and its 3-methoxy derivative (MHPG), and the recent demonstration in our laboratory that MHPG can be converted to VMA in Man, has stimulated studies of these compounds in experimental animals.

Using tertiary-butyl-dimethylsilyl derivative, methods have been developed to assay by gas-chromatography-mass spectroscopy levels of MHPG and DHPG in small volumes of plasma. Pharmacological blockade of reuptake of norepinephrine(NE) into sympathetic nerves diminishes stimulation-induced increments in plasma DHPG and MHPG (Jimerson, Yamaguchi and Kopin). When prostaglandin enhances stress-induced increases in plasma NE, the metabolites are also increased (Jimerson, Feuerstein and Kopin). These results suggest that the metabolites are formed from released NE which is recaptured by the sympathetic nerves.

Conversion of MHPG to sulfate and glucuronide conjugates in rats is the major metabolic fate of MHPG, but a small amount of MHPG is converted to VMA (Lozovsky and Kopin), but in man, oxidation to VMA is a major metabolic route. Inhibition of sulfate conjugate formation by administration of dinitrophenol increases glucuronide formation with only slight effects on MHPG conversion to VMA. Even in Gunn rats, which have a genetic lack of glucuronide formation, inhibition of sulfate conjugation enhanced only slightly oxidation of MHPG to VMA. This indicates that the alcohol dehydrogenase responsible for oxidation of the glycol is not an effective enzyme in rats and preferential formation of conjugates of MHPG in rats is not a result of greater efficiency of conjugation.

PUBLICATIONS:

Jimerson, D.C., Sun, C.L., Yamaguchi, I. and Kopin, I.J.: Plasma levels of glycol metabolites and presynaptic metabolism of norepinephrine (NE). In Usdin, E., Kopin, I.J. and Barchas, J. (Eds.) Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, pp 927-929, 1979.

Yamaguchi, I. and Kopin, I.J.: Blood pressure, plasma catecholamines and sympathetic outflow in pithed SHR and WKY rats. Am. J. Physiol. H365-H372, 1980.

McCarty, R., Chiueh, C.C. and Kopin, I.J.: Differential Behavioral Responses of Spontaneously Hypertensive (SHR) and Normotensive (WKY) Rats to d-Amphetamine. Pharmacol. Biochem. & Behav. 12: 53-59, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00402-08 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

CNS Regulation of Autonomic and Endocrine Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Gertrude Maengwyn-Davies	Guest Worker	LCS NIMH
	Nguyen B. Thoa	Pharmacologist	LCS NIMH
	Marta Weinstock	Visiting Scientist	LCS NIMH
	Anthony Zavadil	Guest Worker	LCS NIMH
	Charles Saller	Guest Worker	LCS NIMH

COOPERATING UNITS (if any)

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Section on Pharmacology

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to define the regions of brain, the neuronal pathways, and the neurotransmitter systems which control endocrine, metabolic and autonomic responses to internal stimuli (e.g., blood glucose, O_2-tension, hormonal state, body temperature, etc.) and to various types of stress (immobilization, foot-shock, hemorrhage, etc.), and the effects of drugs on these systems.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The central nervous system integrates information received from various chemo-and baro-receptors and other sensors of the internal environment and appropriately modulates various respiratory and cardiovascular functions. Using techniques of micro-injection, of drugs on putative neurotransmitters, microdissection and microassay of neurotransmitters and their metabolites, destruction of specific areas of brain, or determination of glucose utilization in different regions, the physiological roles of various substances and/or particular nuclei or tracts can be examined.

Prostaglandins of the F_2 series elevate blood pressure and heart rate an action mediated through the central nervous system. Specific forebrain nuclei which appear to be involved in eliciting these responses have been identified and are being explored (Feuerstein, Jacobowitz and Kopin).

Dopaminergic neurones in brain appear to be sensitive to changes in blood glucose levels, decreasing their firing rate and releasing less dopamine (as indicated by a decrease in dopamine metabolites) when blood glucose is elevated (Dr. Saller).

Even small amounts of glucose, comparable to the amounts consumed during a meal or released by the liver during stress, suppress both the firing of dopaminergic neurons in the brain and central dopamine release. In addition, glucose administration can modify the response of central dopaminergic neurons to the antipsychotic agent, haloperidol. Haloperidol administration normally produces a large increase in impulse flow along dopaminergic axons and activates tyrosine hydroxylase, thought to be the rate-limiting enzyme in catecholamine synthesis. However, glucose administration can either prevent or suppress the increased firing of dopamine-containing neurons normally ellicited by haloperidol, but it does not prevent tyrosine hydroxylase activation. The latter finding suggests that haloperidol and perhaps other antipsychotic drugs may influence central dopamine metabolism independently of their effects on impulse flow.

A simple yet sensitive and specific assay for the analysis of O-methylated catecholamines in both tissues and urine has been developed and is being applied to measure central and peripheral catecholaminergic activity in animals and human subjects (Saller, Oddershede and Lozovsky). Conjugates of O-methylated amines have been found in rat brain.

The C^{14}-2-deoxyglucose method has been used in an attempt to map the baroreceptor pathway in the brainstem of the pentobarbital-anethetized cat (DiMicco, Sokoloff and Kopin). Preliminary experiments suggest that electrical stimulation of one carotid sinus nerve (which results in activation of the baroreceptor pathway as evidenced by a fall in arterial pressure and heart rate) produces an asymmetric pattern of enhanced glucose uptake in areas of the brain thought to be involved in cardiovascular regulation. Glucose uptake was greater in the ipsilateral nucleus of the tractus solitarius (NTS) at the level of the obex (first synapse of primary baroreceptor afferents in the central nervous system) and in the nucleus ambiguous (secondary projection of the NTS and site of origin of cardiac vagal preganglionic fibers) than in the corresponding structures on the side contralateral to nerve stimulation.

Oxotremorine is a muscarinic cholinergic drug which produces tremor in rats. The drug induces increases in dopamine (DA) turnover and thereby elevates levels of homovanillic acid (HVA) in the Corpus Striatum (C.S.) and N. Accumbens (N.A.). Both propranolol (a β-receptor blocking agent) and clonidine (an α-receptor stimulator) diminish the oxotremorine-induced increases in HVA in the C.S. but not in the N.A. Haloperidol, which blocks DA receptors also increases levels of HVA in the C.S. and N.A., but its effects are not blocked by either propranolol or clonidine. These results indicated that norepinephrine may mediate the cholinergic stimulation of release of DA in the C.S. but not in the N.A. (Weinstock, Zavadil, Kopin).

PUBLICATIONS:

Weinstock, M., Zavadil, A.P. III, and Kopin, I.J.: Differential effects of d- and l-propranolol on dopamine turnover stimulated by oxotremorine in striatal and mesolimbic areas of rat brain. Eur. J. Pharmacol. 59: 187-193, 1979.

Kopin, I.J.: Mode of Action of: Antidepressants and Central Stimulants. Chapter to Handbook of Biological Psychiatry Vol.II in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00403-07 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Control of Catecholamines and DBH in Human Plasma and CSF

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
	C. Raymond Lake	Staff Psychiatrist	LCS NIMH
OTHER:	Virginia K. Weise	Chemist	LCS NIMH
	Michael D. Coleman	Nephrologist, Holt Krock Med. Clin., Fort Smith, Ark.	
	Sally Anderson	Biologist	LPP NIMH
	David Lozovsky	Psychiatrist	LCS NIMH
	Edna K. Gordon	Chemist	LCS NIMH
	Young Kim	Anesthesiologist	CC ANES

COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS; Holt Krock Medical Clinic, Fort Smith, Arkansas; Dept. of Medicine, Mt. Sinai Hospital, New York, N.Y.; Surgical Neurology Branch (NINCDS), NIH, Bethesda, Maryland

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine (DA), norepinephrine (NE), and epinephrine (EPI) and their metabolites and dopamine-β-hydroxylase (DBH) are present in human plasma and cerebrospinal fluid (CSF). Levels of these substances reflect the balance between their release and removal. The increases in levels of NE and EPI with standing, exertion, stress, drugs, administration, etc., are used to examine sympathetic neuronal responsivity. The responses and level of activity of the sympathetic nervous system in cardiovascular disorders (hypertension, hypotension) in alcoholism, a variety of psychiatric disorders, and after treatment with drugs are being examined. Dopamine-beta-hydroxylase (DBH) is the enzyme which converts dopamine (DA) to NE. NE and DBH can be measured in cerebrospinal fluid (CSF) and may be useful to estimate in diseases in which a defect is suspected, i.e., essential hypertension, Shy-Drager syndrome, amyotrophic lateral sclerosis (ALS), enuresis and hyperactivity in children, and schizophrenia.

PROJECT DESCRIPTION:

Blood pressure is maintained by changes in sympathetic neuronal activity. These changes are mediated by the central nervous system which receives information from baroreceptors in the aorta and carotid sinus and volume receptors in the right atrium. Plasma levels of norepinephrine(NE) reflect acute changes in sympathetic neuronal activity because the catecholamine is rapidly metabolized and its levels are maintained by continuous secretion into the blood. Dopamine-β-hydroxylase (DBH) in plasma is derived from sympathetic nerves, but its levels are genetically determined and change slowly in any individual because it is slowly metabolized. Thus, in a single individual, changes in levels of DBH reflect chronic changes in sympathetic activity or in fluid distribution, but genetic differences account for most of the differences in DBH of plasma from different persons.

In our studies, as well as in several other reports, levels of NE in hypertensive patients are similar to those in normal subjects, but the relationship of NE levels in plasma to age found in normal subjects is not apparent in the hypertensive population, because among the hypertensive subjects there are a few young patients with high levels of plasma NE. In other studies, hypertensive patients have been found to have higher levels of plasma NE than in normotensive subjects. In these studies the relationship of plasma NE levels to age was not found. Analyses of the results of both groups of studies shows that the difference between the two groups is a result of a difference in the normal subjects. Studies in which hypertensives were found to have higher levels of plasma NE than in normal subjects had values for normal controls which were significantly lower, and had lower variance, than did the normotensive subjects in studies in which hypertensives and normotensive subjects had similar levels of plasma NE. This difference may be attributed to use of a biased group of older normal subjects or because most patients studied were in the younger age groups consisting of medical personnel who were familiar with hospital procedures found in random normotensive and hypertensive subjects.

Decreases in plasma volume, as a result of treatment with diuretics, in normal or hypertensive patients, evoke reflex-mediated increases in sympathetic activity reflected by increased levels of plasma NE, but not DBH. The enhanced sympathetic activity and elevation in plasma levels of NE attending treatment of hypertensive patients with drugs which block actions of catecholamines, produce vasodilation, or diminish vascular volume, can be prevented by administration of drugs, such as clonidine or α-methyl dopa, which activate α-adrenergic receptors in the central nervous system. Fenfluramine, a drug which interacts with serotonergic neurones, also diminishes plasma catecholamines by an unknown mechanism involving the central nervous system.

In children, imipramine and dimethylimipramine elevate blood pressure and increase plasma levels of norepinephrine, while in adults this effect is not seen. Possibly the differences between adults and children are the relatively poorly developed baroreceptor reflexes in children.

Increased levels of NE are evoked by stress, sexual excitation, hypoglycemia, and volume depletion during hemodialysis for chronic renal failure. In patients with Down's syndrome plasma NE levels are normal and respond normally to stress, but levels of DBH in plasma are low, perhaps on a genetically related basis.

The ability to assess directly noradrenergic neurotransmission in brain areas adjacent to the cerebral ventricles by assaying NE from CSF yields information about nerve function and alterations in various disease conditions or with the administration of various drugs. Some patients with essential hypertension or schizophrenia have elevated levels of NE in CSF.

PUBLICATIONS:

Kopin, I.J.: Plasma catecholamines in human and experimental hypertension. In Meyer, P. and Schmitt, H. (Eds.): Nervous System and Hypertension, New York, John Wiley & Sons, 1979 (pp. 267-276).

Kopin, I.J.: Central nervous system actions of anti-hypertensive drugs. Drug Therapy 4: 30-36, 1979.

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Lake, C.R.; Ziegler, M.G.; Coleman, M.D. and Kopin, I.J.: Plasma levels of norepinephrine and dopamine-β-hydroxylase in CRF patients treated with dialysis. Cardiovasc. Med. 4: 1099-1111, 1979.

Kim, D.Y., Lees, D.E., Lake, C.R., Whang-Peng, J., Schuette, W., Smith, R., and Bull, J.: Acute hyperthermic potentiation of adriamycin cardiotoxicity. JAMA 241: 1816-1817, 1979.

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Lake, C.R., Coleman, M.D., Ziegler, M.G., and Murphy, D.L.: Fenfluramine and its effects on the sympathetic nervous system in man. Curr. Med. Res. Opin. 6: 63-72, 1979.

Ziegler, M.G., Lake, C.R., Williams, A.C., Teychenne, P.F., Shoulson, I., and Steinsland, O.: Bromocriptine inhibits norepinephrine release. Clin. Pharm. Ther. 24: 137-142, 1979.

Ziegler, M.G. and Lake, C.R.: Norepinephrine alterations associated with physiologic and pathologic activity of central sympathetic nervous system in man. In wood, J.H. (Ed.): Neurobiology of Cerebrospinal Fluid. New York, Plenum Press, 1980 (in press).

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Roizen, M.F., Moyers, J.R., Zivin, J.A., Wilkinson, P.L., Lake, C.R., Hamilton, W.K., Chatterjee, K., and Horrigan, R.W.: Predictability of the change in plasma norepinephrine concentration in one vascular bed knowing the change in plasma norepinephrine in another vascular bed. In Usdin, E., Kopin, I.J. and Barchas, J. (Eds): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, Vol 1, 1979, pp 930-932.

Wiedeking, C., Ziegler, M.G. and Lake, C.R.: Plasma noradrenaline and dopamine-beta-hydroxylase during human sexual activity. J. Psychiatr. Res., 15: 139-145, 1979.

Kopin, I.J., McCarty, R. and Yamaguchi, I.: Plasma Catecholamines in Human and Experimental Hypertension. Clin. Exp. Hypertens. 2(3,4): (in press), 1980.

Kopin, I.J., Goldstein, D. and Feuerstein, G.: The Sympathetic Nervous System and Hypertension: A position paper. Symposium: Frontiers in Hypertension Research, N.Y. Hospital, Cornell Medical Center. 1980 (in press).

Kopin, I.J.: Catecholamines and Blood Pressure Regulation. In Soffer, R.L. (Ed.): The Biomedical Regulation of Blood Pressure. New York, John Wiley & Sons, 1980 (in press).

Lake, C.R., Ziegler, M.G., Coleman, M.D. and Kopin, I.J.: Fenfluramine potentiation of thiazide antihypertensive effects. Clin. Pharmacol. Ther. 1980 (in press).

Kim, D.Y., Lake, C.R., Lees, D.E., Schuette, W.H., Bull, J.M., Weise, V. and Kopin, I.J.: Hemodynamic and plasma catecholamine responses to hyperthermic cancer therapy in humans. Amer. J. Physiol. 1979, Vol. 237 (5), pp. M570-574.

Lake, C.R., Sternberg, D.E., Van Kammen, D.P., Ballenger, J.C., Ziegler, M.G., Post, R.M., Kopin, I.J. and Bunney, W.E.: Schizophrenia: Elevated cerebrospinal fluid norepinephrine. Science, 1980, Vol. 207 (4428), pp. 331-333.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00404-09 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Growth Characteristics and Immunological Localization of Enzymes in Neurones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Gad Gilad	Visiting Fellow	LCS NIMH
	Wolfgang Oertel	Guest Worker	LCS NIMH
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	Marcel Tappaz	Visiting Fellow	LCS NIMH
	Enrico Mugnaini	Chief Lab. of Neuromorphology	State Univ. of Connecticut, Storrs, Conn.

COOPERATING UNITS (if any)

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SECTION
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NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunohistological methods are being used to define the distribution in the nervous system and other tissues of cells (neurones, glia, etc.) which contain specific enzymes associated with particular functions (e.g., production of specific neurotransmitters, hormones, etc.). The ontogeny of the specific proteins and their role in growth, differentiation and regenerative processes are being examined using these techniques.

PROJECT DESCRIPTION:

Studies of enzyme levels during development of the rat cerebellum showed differences in the patterns of appearance of tyrosine hydroxylase, glutamine acid decarboxylase, and choline acetylase (enzymes which control synthesis of dopamine and norepinephrine, α-aminobutyric acid (GABA), and acetycholine, respectively). These enzymes reflect development of specific neuronal systems. The enzymes which control polyamine synthesis, ornithine decarboxylase and S-adenosylmethionine decarboxylase, increased transiently and declined to low levels in the adult, perhaps reflecting the role of polyamine in initiating different phases of development. After axonal injury, changes in enzyme levels in the areas of the cell bodies reflect a shift in synthesis of proteins from those concerned with structural aspects rather than those involved in neurotransmitter formation.

Two-dimensional electrophoresis has been used to separate the enzymes which are irreversibly inhibited with radioactivity labeled "Suicide Inhibitors". Glutamic acid decarboxylase (GAD) has been purified by similar techniques and used to stimulate an antibody to the enzyme.

The antibody to glutamic acid decarboxylase (GAD), the biosynthetic enzyme for the major inhibitory neurotransmitter GABA, was raised and characterized. Using immunocytochemical techniques on the light - and electronmicroscopic level GAD-immunoreactivity was localized specifically in different brain areas including the cerebellum, olfactory bulb, cerebral cortex and basal ganglia in rat brain. GAD-immunoreactivity was also investigated in specific brain areas after injection of neurotoxic agents and selective neurosurgery in order to reveal GABAergic pathways. Future studies will concentrate on the functional anatomy of the GABAergic system of the rat and characterization of GABAergic components in different tissue culture systems.

Glutamic acid decarboxylase was compared with the enzyme activity of cysteine sulfinic acid decarboxylase (CSD), the proposed biosynthetic enzyme for hypotaurine and taurine. CSD activity was separated in two enzyme activities, termed CSD I and CSD II. CSD I was found to be extremely similar to liver CSD, but CSD II was indistinguishable from GAD by biochemical and immunological techniques. Subsequent work will test whether CSD I is a glial enzyme or is present in neurones.

PUBLICATIONS:

Gilad, G.M., Gagnon, C., and Kopin, I.J.: Protein carboxymethylase activity in the rat superior cervical ganglion during development and after axonal injury. Brain Res. 183: 393-402, 1980.

Gilad, G.M. and Kopin, I.J.: Neurochemical aspects of neuronal ontogenesis in the developing rat cerebellum: Changes in neurotransmitter and polyamine synthesizing enzymes: J. Neurochem. 33: 1195-1204, 1979.

Gilad, G.M., Gilad, V.H. and Kopin, I.J.: The reaction of the mutant mouse nude to axonal injuries of the central nervous system: Exp. Neurol 65: 87-98, 1979.

Oertel, W.H., Tappaz, M.L., Kopin, I.J., Ransom, D.H. and Schmechel, D.E.: Antiserum to rat brain glutamate - cysteine sulfinate decarboxylase: Brain Res. Bulletin (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00405-01 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study of Aminergic Receptor Function In Vivo

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief	LCS NIMH
Other:	Joseph DiMicco	Staff Fellow	LCS NIMH
	Giora Feuerstein	Visiting Associate	LCS NIMH
	Tichomir Torda	Visiting Fellow	LCS NIMH
	Isamu Yamaguchi	Guest Worker	LCS NIMH
	Candace Pert	Pharmacologist	BP NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Medicine

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to assess changes in properties (number, affinity) of receptors in response to alterations in the environment, drug administration, stress, or hormones, and to develop methods for assessing receptor function in intact animals, in vivo. Stress or isoproterenol-induced reduction of adrenoceptors can be prevented by administration of quinacrine. The α-adrenoceptor mediated pressor responses to administered norepinephrine are of the α_2 type whereas different α_1-adrenoceptors (in the junction) respond to norepinephrine released from sympathetic nerves. Labelling of β-receptors in vivo provides a means of measuring receptors in intact animals and humans.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

In rats, forced immobilization or repeated injections of isoproterenol results in subsensitivity of β_1-adrenoceptors and α-adrenoceptors. This has been demonstrated by showing that the numbers of receptors are decreased (Torda, Yamaguchi, Hirata, Kopin and Axelrod). There is a parallel decrease in the cardiac acceleration and increase in blood pressure produced by administered catecholamines. These changes in receptor density and responsiveness to catecholamines can be prevented by administration of quinacrine, an antimalarial agent which blocks phospholipase A_2. These studies indicate that the effects of quinacrine of phospholipid turnover alter the changes in receptor density and this action is physiologically relevant to adrenergic receptor function.

Examination of the effects of different α-adrenoceptor blocking agents on the pressor responses to sympathetic stimulation or to administered norepinephrine in pithed rats and comparison with the effects of these drugs on stimulation-evoked increases in plasma catecholamines has shown that α_1-type adrenoceptors mediate the pressor response to norepinephrine released at sympathetic nerve endings whereas, β_2-type adrenoceptors are responsible for the pressor effect of administered norepinephrine as well as presynaptic feedback regulation of norepinephrine release. The implication of these observations is that α_1-adrenoceptors are located at the junction of the sympathetic nerves with the post-synaptic membrane while α_2-receptors are extrajunctional (Yamaguchi and Kopin).

After intravenous administration of a trace amount of ^{3}H-dihydroalprenolol to rats, some of this labelled β-adrenoceptor blocking agent is bound to receptors. The labelled compound can be displaced by administration of pharmacologically significant amounts of alprenolol or other β-blocking agents. The displacement is sterospecific and appears to derive mainly from β_2-type receptors, perhaps in the lung (DiMicco, Kopin and Pert).

PUBLICATIONS:

DiMicco, J.A., Kopin, I.J., and Pert, C.B.: Evaluation of β-adrenergic receptor status by plasma sampling in living rats. Proceedings on Conference on the Psychopharmacology and Biochemistry of Neurotransmitter Receptors, Elsevier North Holland, Inc. (in press).

Yamaguchi, I. and Kopin, I.J.: Differential Inhibition by α-Adrenergic Antagonists of α_1- and α_2-Adrenoceptors in Pithed Rats. J. Pharmacol. Exp. Ther., 1980 (in press).

Kopin, I.J.: Assessment of Adrenergic Receptors In Vivo in Experimental Animals and Man. Proceedings on Conference on the Psychopharmacology and Biochemistry of Neurotransmitter Receptors, Elsevier North Holland, Inc. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00378-06 LCS

PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Immunofluorescence Study of the Uptake of the Antibody to Dopamine-β-Hydroxylase in the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Daniel A. Pasquier	Guest Worker	LCS	NIMH
	Mark A. Gold	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A retrograde flow mapping technique that is specific for dopamine-β-hydroxylase (DβH) containing neurons was used to trace the cell body origins of noradrenergic neuronal projections to the cerebellum. Following unilateral or midline injection of the antibody to DβH (ADβH), noradrenergic cell bodies were observed to contain the ADβH in the pontine tegmentum. This work demonstrates that in addition to the well known locus coeruleus projection to the cerebellum, projections from the subcoeruleus, A5 and A7 groups of cells exist. All cell groups projected both ipsilaterally and contralaterally.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The elucidation of the origin of noradrenergic cell bodies which project to the cerebellum.

Methods Employed: Immunohistochemical localization of ADβH by an indirect immunofluorescence technique utilizing a fluorescein-labelled antiserum to the primary antibody. The rats received unilateral and vermal stereotaxic injections of ADβH via a glass micropipette.

Major Findings: The major noradrenergic nerve input to the cerebellum has its origin in the caudal part of the locus coeruleus. In addition, a few subcoeruleus A5 and A7 cells were seen bilaterally. No cell bodies were observed in the A1 or A2 cell groups.

Significance to Biomedical Research and the Program of the Institute: This study represents the first indication that some noradrenergic fibers which innervate the cerebellum arise from the subcoeruleus, A5 and A6 cell bodies in addition to the locus coeruleus. The functional significance of multiple noradrenergic projections to the cerebellum is currently unknown.

Publication:

Pasquier, D.A., Gold, M.A. and Jacobowitz, D.M.: Noradrenergic perikarya (A5-A7, subcoeruleus) projections to the rat cerebellum. Brain Research, in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00382-06 LCS

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical Mapping of Neurotransmitter Neurons of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharmacology	LCS	NIMH
	Samuel Speciale	Guest Worker	LCS	NIMH
	Zehava Gottesfeld	Guest Worker	LCS	NIMH
	Motohiro Kato	Guest Worker	LEN	NINCDS
	Raymond R. O'Neill	Biologist	LEN	NINCDS
	William F. Caveness	Chief	LEN	NINCDS

COOPERATING UNITS (if any)

Laboratory of Experimental Neurology, NINCDS

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: 1.6	PROFESSIONAL: 1.3	OTHER: .3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A discrete regional analysis of norepinephrine (NE), dopamine (DA), choline acetyltransferase (CAT) and glutamic acid decarboxylase was performed in the brain of the newborn and pubescent monkey. A method is described for the removal of discrete areas of the monkey brain. Comparison between newborn and pubescent monkeys showed 3 - 4.5 fold increase in DA in the caudate and putamen of the pubescent monkeys and a 1.5 - 4.0 increase in CAT activity in most areas. The GAD activity was generally lower in the newborn when compared to the pubescent brain except for the sensory cortex and parts of the basal ganglia which showed a significant increase.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To map biochemically, the norepinephrine (NE), dopamine (DA), choline acetyltransferase (CA) and glutamic acid decarboxylase in the brain of the newborn and pubescent monkey.

Methods Employed: 1. A new method was described for the removal of discrete areas of the monkey brain. The frozen head of the monkey was incased in a cellulose block and was serially sectioned in a horizontal plane with a PMV cryomicrotome which is capable of cutting through the bony cranium and the brain tissue. The brain areas and nuclei of interest were identified on the surface of the brain according to stereotaxic atlases and small samples of discrete regions of the brain were removed by the use of stainless steel cannulae. 2. Microradioenzymatic assays for NE, DA, CAT, GAD.

Major Findings: There were no significant differences in NE concentrations in various regions of the newborn and pubescent brains. The DA concentrations in the caudate and putamen were very high and were 3-4.5 fold greater in the pubescent than in the newborns. In the newborn CAT activity was generally 1.5-4.0 times lower than in the pubescent animal. GAD activity in the newborn was generally lower than the pubescent monkey brain. Significant increases in the pubescent to newborn ratio was observed in the sensory cortex, parts of the caudate and putamen, the dorsolateral part of the globus pallidus, subthalamic nucleus, substantia nigra reticulata, cerebellum and nucleus interpositus.

Significance to Biomedical Research and the Program of the Institute: The present methodology lays the groundwork for future work that will require analysis of neurotransmitters, enzymes and various neuroactive agents in the brain of large animals.

Publications:

Jacobowitz, D.M., Kato, M., O'Neill, R.R., Speciale, S.G., Gottesfeld, Z. and Caveness, W.F.: Discrete regional analysis of norepinephrine, dopamine, choline acetyltransferase and glutamic acid decarboxylase in the brain of the newborn and pubescent monkey. Brain Research Bulletin, in press, 1980.

O'Donohue, T.L., Crowley, W.R. and Jacobowitz, D.M.: Biochemical mapping of the noradrenergic ventral bundle projection sites: Evidence for a noradrenergic-dopaminergic interaction. Brain Research 172: 87-100, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00388-04 LCS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neurochemical and Histochemical Studies of Cholinergic Pathways of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Cinda J. Helke	Guest Worker	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH
	Mark Gold	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: .6	OTHER: .9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the rat brain changes in choline acetyltransferase (CAT) activity following stereotaxic lesions revealed that cholinergic nerves of the nucleus of the tractus diagonalis project to the cortex (frontal, cingulate, entorhinal, occipital), hippocampus, amygdala, dorsomedial nucleus, lateral habenula, globus pallidus, anterior ventral nucleus of the thalamus and interpeduncular nucleus. The nucleus tractus diagonalis is a major cholinergic nucleus that projects long descending neurons to regions of the forebrain, midbrain and hindbrain.

PHS-6040
(Rev. 10-76)

Objectives: To investigate possible cholinergic projection sites of innervation from the tractus diagonalis of the rat brain.

Methods Employed: 1. Bilateral stereotaxic lesion of the nucleus tractus diagonalis. 2. Microdissection of brain nuclei. 3. Radioenzymatic assay for choline acetyltransferase (CAT).

Major Findings: Following stereotaxic lesions of the nucleus tractus diagonalis significant decreases in CAT activity were found in the cortex (frontal, cingulate, entorhinal, occipital), hippocampus, amygdala (medial, basalis), dorsomedial nucleus, lateral habenula, globus pallidus, anterior ventral nucleus of the thalamus and interpeduncular nucleus.

Significance to Biomedical Research and the Program of the Institute: The nucleus tractus diagonalis appears to be an area of major projections of cholinergic nerves in the brain. This nucleus is analogous to the locus coeruleus projection of noradrenergic nerves. This is important for studies aimed at determining functions of acetylcholine in the brain.

Proposed Course of Project: Work has been initiated to purify choline acetyltransferase in order to produce an antibody which will enable complete mapping of cholinergic nerves in the brain.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00389-04

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Ovarian Hormones in Plasma Gonadotropins and on Serotonin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .6	PROFESSIONAL: .4	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

TERMINATED

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00394-03 LCS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Role of Central Cholinergic Mechanism in the Feedback Actions of Gonadal Steroids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .9	PROFESSIONAL: .7	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous work on this project established a feedback effect of gonadal hormones on the cholinergic activity of the n. tractus diagonalis (td) and the ventral tegmental area (avt) of the rat brain, concomitant with hormone-induced alterations in luteinizing hormone (LH) secretion. The present work suggests that a) the changes in cholinergic activity are secondary to the alterations in LH secretion, rather than causative; b) the changes may in fact result from hormone-induced alterations in pituitary trophic hormone secretion, since hypophysectomy prevents them; c) the observed changes in cholinergic activity are a physiological manifestation of a neuroanatomical link between the mesolimbic dopamine (DA) system originating in the avt and the cholinergic system originating in the td.

Project Description:

Objectives: a) To establish whether the hormone-induced alterations in cholinergic activity of the avt and the td are causative or secondary to the concomitant alterations in pituitary LH secretion; b) to delineate the functional interaction between the mesolimbic dopamine system originating in the avt and the cholinergic system originating in the td.

Methods Employed: 1) Surgical castration. 2) Surgical hypophysectomy. 3) Stereotaxic ablation and electrical stimulation of the td and avt. 4)Stereotaxic DA, 6-OHDA, and testosterone implantation in the td. 5) Cannulation of tail and femoral vein for drawing blood. 6) Systemic testosterone injection. 7) Focussed microwave irradiation of heads of animals used for ACh microassay. 8) Radioimmunoassay of LH in plasma. 9) Microdissection of brain nuclei. 10) Microenzymatic assays for ChAT, ACh, DA, and NE. 11) Glyoxylic acid-induced catecholamine histofluorescence of unfixed frozen sections (de la Torré method).

Major Findings: 1) In male rats, ablation of the td or the avt failed to prevent the castration-induced rise in LH. Furthermore, neither electrical stimulation of the td or the avt in both sexes nor implantation of DA in the td of males (cf #2 below) had any significant effect on plasma LH titers. It was therefore concluded that the observed hormone-induced cholinergic responses in these areas were secondary to the hormone-induced alterations in LH secretion. 2) Ablation of the avt, including the mesolimbic A10 DA cell bodies, resulted in a 75% decrease in DA content of the td. The td is therefore among the forebrain regions innervated by this mesolimbic DA system. 3) Unilateral 6-OHDA implantation in the td, while having no local effect, caused a 30% decrease in the ChAT activity of two putative projection areas, namely the contralateral td and the ipsilateral avt. This suggests stimulatory effect of catecholamine terminals innervating the td ACh cell bodies. 4) Implantation of crystalline testosterone propionate in the td, while causing a slight but significant decrease in ChAT activity there, had no effect on the ChAT activity of several known td cholinergic projection areas. Furthermore, hypophysectomy prevented the effect of gonadal hormone manipulation on the ChAT activity of the td. Taken together, these results suggest that the effects seen in the td cholinergic activity after gonadal hormone manipulation result from the altered action of LH on the td rather than a direct action of the gonadal hormone on the td. 5) Over the course of this project, both the DA content and the ChAT activity of the n. tractus diagonalis have changed several fold. In addition, the effect of systemic testosterone injections on the td ChAT activity of castrated rats in a recent experiment was the opposite of the effect in a previous, identical experiment. These events have occurred despite vigorous attempts to control for diurnal variations, and are suggestive of some other rhythm, as yet unknown.

Significance to Biomedical Research and the Program of the Institute: This study provides insight into how circulating hormones may affect behavior by elaborating their effects at the level of the individual nuclei of the brain. At the same time, it explores the functional interaction of the td cholinergic system with the mesolimbic DA system. The elucidation of this interaction at the neurochemical/neuropharmacological level, while advancing our understanding of neuroendocrinology, may in addition help to explain the effects of the neuroleptics and other psychotropic drugs known to exert their effects *via* dopaminergic and cholinergic systems.

Proposed Course of Project: 1) The DA/ACh interaction mentioned above will be further explored by examining the effects of heloperidol and avt stimulation on td ChAT activity and ACh content. 2) A diurnal study will be undertaken to determine the cyclicity of DA and ChAT in the td, in order to examine the possibility of a seasonal shift in such a rhythm or the existence of a seasonal rhythm itself.

Publication:

Muth, E.A., Crowley, W.R., Jacobowitz, D.M.: Effect of gonadal hormone manipulation on plasma luteinizing hormone and on choline acetyltransferase activity and acetylcholine levels in discrete nuclei of the rat brain. *Neuroendocrinology* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00395-02 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Choline Acetyltransferase Activity in DOCA-Salt Hypertensive Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Cinda J. Helke	Guest Worker	LCS	NIMH
	Bryan B. Sohl	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology Section

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .7	PROFESSIONAL: .4	OTHER: .3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The choline acetyltransferase (ChAT) activity was measured in numerous discrete brain nuclei of DOCA-Salt hypertensive rats. Three control groups were employed. These rats received either 1) normal drinking water, no DOCA, 2) saline (1%) drinking water, no DOCA 3) normal drinking water plus DOCA.

Changes in the ChAT activity of the dorsomedial nucleus of the hypothalamus (20% decline) and the intermediate portion of the nucleus tractus solitarius (31% increase) were found in the DOCA-salt hypertensive rats compared to the normotensive control rats. In addition, a decline in the ChAT activity of the supraoptic nucleus was noted in the 2 groups of salt treated rats. The former changes are likely related to the elevated BP while the latter appear to be a response to chronic salt treatment rather than elevated BP.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Recent pharmacological studies have demonstrated that CNS cholinergic mechanisms may be involved in blood pressure control. In addition, we have shown that biochemical differences in cholinergic neurons of discrete brain areas exist between spontaneously hypertensive rats and their normotensive controls. The present study is an attempt to 1) investigate CNS cholinergic neurons in another rat model of hypertension and 2) to assess whether or not the changes seen in the spontaneously hypertensive rats were merely a response to the elevated BP as the DOCA-salt rats will achieve a similar degree of BP elevation but the mechanism is different.

Methods Employed: 1) Indirect tail cuff measurement of systolic blood pressure. 2) Microdissection technique for removal of discrete nuclei. 3) Radioisotopic assay for choline acetyltransferase.

Major Findings: 1) The systolic BP of DOCA-salt rats reached 185 mmHg after 4 weeks of treatment, a level identical to that seen with the 12 week old spontaneously hypertensive rats (SHR) used in the previous study. 2) As was seen in the SHR rats a significant decline in the choline acetyltransferase activity was found in the dorsomedial nucleus of the hypothalamus, thus the change is likely to be a secondary response to the elevated BP rather than a causative factor. 3) An alteration in the ChAT activity of the nucleus tractus solitarius was also found in the DOCA-salt hypertensive rats. 4) The activity of ChAT in the supraoptic nucleus was decreased by chronic salt treatment but was unrelated to the elevated BP of the DOCA salt group as the normotensive control group which received salt but not DOCA also exhibited this change.

Significance to Biomedical Research and the Program of the Institute: Evidence has been provided that CNS cholinergic neurons are altered in DOCA salt hypertension as well as in SHR. Certain of these changes (i.e. dorsomedial hypothalamus) may be merely a response to the elevated BP as they are evident in the hypertensive rats of each model. However others are unique to each model and may therefore be involved in the development and/or maintenance of the hypertension. These studies and future studies may eventually enhance our knowledge of CNS mechanisms of cardiovascular control and hypertension.

Proposed Course: Terminated.

Publication:

Helke, C.J., Sohl, B.D. and Jacobowitz, D.M.: Choline acetyltransferase activity in discrete brain nuclei of DOCA-salt hypertensive rats. Brain Res. in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00396-02 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies on Substance P as the Neurotransmitter of Baro- and Chemoreceptor Afferents in the Nucleus Tractus Solitarius

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Nguyen B. Thoa	Pharmacologist	LCS	NIMH

COOPERATING UNITS (if any)

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Laboratory of Clinical Science

SECTION
Section on Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .9	PROFESSIONAL: .7	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Further evidence that substance P (SP) is a neurotransmitter of baro- and/or chemoreceptor afferent nerves has been obtained by 1) measuring the (SP) content of the nucleus tractus solitarius (NTS) in the rat following unilateral nodose ganglionectomy 2) utilizing immunocytochemistry to detect the presence of SP in peripheral baro- and chemoreceptive regions and pathways of the afferent fibers and 3) investigating the potassium stimulated release of SP from the NTS *in vitro*. It was found that the NTS contained significant amounts of SP-I and that unilateral removal of the nodose ganglia reduces the SP-I content of those portions of the NTS known to receive vagal afferents. In addition, SP-I was visualized in discrete fibers in the tunica adventitia of the aortic arch and carotid sinus regions, the carotid body, the vagus nerve and nodose ganglia. Stimulation of freshly dissected slices of the NTS region with 50 mM potassium in the presence of calcium increases SP release 2-3 fold. Omission of calcium from the buffer completely abolished SP release by K^+. These results in the rat are consistent with our previous studies in the cat and provide further evidence that SP is contained within baro- and chemoreceptor afferent nerves.

Project Description:

Objectives: Various investigators using immunohistochemical techniques have reported substance P like immunoreactivity in the NTS and fibers entering the brain stem via the glossopharyngeal nerve. In addition, one of the sensory ganglia-containing cell bodies of baro- and chemoreceptor afferent fibers (the nodose ganglion) has been described as containing substance P immunoreactive cell bodies. Because baro- and chemoreceptor afferents are known to terminate in the NTS the purpose of this study was to determine whether the primary afferent input to the NTS from baro- and chemoreceptors is via substance P neurons. In addition, our previous study in the cat (Brain Res. 181:476, 1980) demonstrated that SP (measured by RIA) was found in the NTS, nodose ganglia and petrosal ganglia and that intracranial removal of the IXth and Xth cranial nerves resulted in a 50% reduction of the SP content of parts of the NTS where baro- and chemoreceptor afferents terminate. The purpose of these studies were to provide further evidence that SP is contained in these nerves by 1) repeating the denervation studies in another species (rat) 2) assess the presence of SP in the peripheral baro- and chemoreceptor region and 3) determine whether SP is released in a calcium dependent manner from the region of the NTS following a depolarizing stimuli.

Methods Employed: 1) Nodose ganglionectomy in rats 2) Microdissection technique for removal of discrete nuclei 3) Radioimmunoassay of substance P 4) HPLC analysis of SP-like immunoreactivity in rat medulla 5) SP immunocytochemistry 6) Stimulation of the release of endogenous SP from rat medullary slices *in vitro.*

Major Findings: 1) Seven to 8d following removal of the left nodose ganglia a significant decline (23%) was noted in the SP content of the left caudal and intermediate portions of the NTS. No significant alterations were found in the nucleus commissuralis or in the rostral NTS. In addition, cervical vagotomy and superior laryngeal nerve section failed to alter the SP content of any region at this time period. 2) SP-I fibers were visualized in the tunica adventia of the aortic arch and fine fibers were found in nerve fascicles as well as in large nerve trunks in proximity to the aortic arch. Distinct SP-I fibers were present in the cervical vagus. In addition, the nodose ganglion contains long varicose fluorescent fibers in the interstitial spaces between cell body clusters. SP fluorescent fibers were seen in the carotid sinus region, in the carotid body and the carotid sinus nerve. 3) SP is released, *in vitro* from slices of the NTS following potassium (50 mM) or capsaicin (3.3×10^{-5}M) induced stimulation in the presence of calcium. Omission of calcium from the buffer (plus 1 mM EGTA) completely abolished simulated SP release from the NTS. The ability of K^+ and capsaicin to release SP from the NTS region supports the hypothesis that SP is a neurotransmitter in afferent nerves projecting to the NTS.

Significance to Biomedical Research and the Program of the Institute: These data suggest that substance P is a neurotransmitter in baro- and chemoreceptor afferents in NTS. This is an important finding in terms of delineating the neurotransmitters in CNS neurons governing cardiovascular reflex activity.

Proposed Course of Project: Additional anatomical studies will be performed to determine whether or not the SP fibers in the aortic arch region are indeed afferent fibers whose cell bodies are located in the nodose ganglia.

Publications:

Gillis, R.A., Helke, C.J., Hamilton, B.L., Norman, W.P. and Jacobowitz, D.M.: Evidence that substance P is a neurotransmitter of baro- and chemoreceptor afferents in nucleus tractus solitarius. Brain Res., 181: 476-481, 1979.

Helke, C.J., O'Donohue, T.L. and Jacobowitz, D.M.: Substance P as a baro- and chemoreceptor afferent neurotransmitter: Immunocytochemical and denervation evidence in the rat. Peptides (in press).

Jacobowitz, D.M. and Helke, C.J.: Localization of substance P immunoreactive nerves. Brain Res. Bull. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00397-02 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of α-Melanocyte Stimulating Hormone in Brain Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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Biology Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology Section

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.1	PROFESSIONAL: 1.9	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a). HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The chemistry, physiology and pharmacology of the α-melanocyte stimulating hormone (α-MSH) system was studied. By combining high pressure liquid chromatography with a radioimmunoassay, α-MSH and deacetylated α-MSH and their respective methionine sulfoxide forms, which are formed artifactually, were identified. By studying the effects of these peptides on behavior, it was determined that deacetylated α-MSH was an inactive form and probably a precursor to α-MSH. α-MSH is released in vitro by veratridine or potassium stimulation in a calcium dependent manner. Stimulation of the central gray causes in vivo release of α-MSH into the cerebrospinal fluid. An acute but not chronic stress also causes a dramatic decrease in brain α-MSH concentrations which is interpreted to indicate a release of α-MSH. A diurnal rhythm of α-MSH in the brain was shifted by clorgyline treatment. α-MSH concentrations in the brain were decreased by fluphenazine treatment and increased by housing rats in chronic dark.

Project Description:

Objectives: Structural identification of α-MSH immunoreactive compounds in the rat and human brain and cerebrospinal fluid and determination of the role of these compounds in the central nervous system.

Methods Employed: 1) α-MSH radioimmunoassay. 2) Microdissection of rat brain nuclei. 3) Push-pull cannulae to remove rat cerebrospinal fluid. 4) Release of α-MSH from hypothalamic slices *in vitro*. 5) High pressure liquid chromatography. 6) Intraventricular injection of α-MSH and observation of grooming and stretch-yawn behavior. 6) Testing of attentional processes after α-MSH administration.

Major Findings: 1) Structural identification of α-MSH-like compounds in the central nervous system. 4 α-MSH immunoreactive compounds were identified in the rat and human brain. 2 compounds coeluted from the high pressure liquid chromatograph with identical retention times to deacetylated α-MSH and α-MSH. The other two peptides coeluted with synthetic α-MSH methionine sulfoxide and deacetylated α-MSH methionine sulfoxide prepared in this lab. The sulfoxide peptides are formed during the extraction procedure. 2) Behavioral activities of the α-MSH-like peptides from rat brain. In order to determine relative potencies of the α-MSH and deacetylated α-MSH, the effect of these peptides on 3 behavioral tests sensitive to MSH-like peptides were studied. Intraventricular administration of 2 μg or 6 μg of α-MSH in the rat produces, in a dose-response fashion, vigorous grooming behavior and the stretch-yawn-syndrome which is characterized by an unusual sequence of vigorous bouts of stretching and yawning. In contrast, deacetylated α-MSH failed to elicit either behavior at either dose. The 6 μg dose of the deacetylated α-MSH sulfoxide was active in inducing the grooming response while α-MSH sulfoxide was inactive. α-MSH and α-MSH sulfoxide also markedly increased the rate of acquisition of a Y-maze visual discrimination task and rats administered α-MSH (40 μg/kg i.p.) learned the task in less than one-half the number of trials required by control rats. Neither the deacetylated α-MSH nor the deacetylated α-MSH sulfoxide were active in the test. These data indicate that the deacetylated form of α-MSH has to be acetylated for activation. It is therefore proposed that an intraneuronal acetylating system may determine the activity of released α-MSH. 3) Release of α-MSH-like peptides *in vitro*. A calcium dependent α-MSH release was identified in rat hypothalamic slices *in vitro*. Release was stimulated by veratridine and inhibited by tetraodotoxin. In contrast, pituitary α-MSH release was inhibited by the presence of calcium. 4) Release of α-MSH into cerebrospinal fluid *in vivo*. To measure the *in vivo* release of α-MSH, rats were perfused through the lateral ventricle with artificial CSF and fractions were collected from the cisterna magna and measured for α-MSH immunoreactivity. Stimulation of the mesencephalic central gray dramatically increased α-MSH concentrations in the CSF while hypophysectomy had no effect. These results indicate that α-MSH in CSF is derived primarily from α-MSH-containing neurons in the brain. 5) Effect of stress on α-MSH concentrations in rat brain. A single immobilization stress resulted in a 40-60% decrease in α-MSH concentrations in the brain regions studied: the medial preoptic nucleus, anterior hypothalamic nucleus, paraventricular nucleus, periventricular nucleus of the thalamus, median eminence, arcuate nucleus and dorsomedial nucleus. This data suggests that stress increased α-MSH release. In contrast, repeatedly immobilized rats had no significant changes in brain α-MSH concentrations suggesting a time or experience related form of neural adaptation. Pituitary α-MSH decreased only slightly after a single but not repeated immobilizations. The increased α-MSH release in stress may reflect a role of α-MSH neural systems in arousal processes. 6) Effects of chlorgyline, fluphenazine and chronic dark on the α-MSH diurnal rhythm in rat brain. Administration of clorgyline to rats housed in a 12 hr light: 12 hr dark cycle resulted in a shift in the brain α-MSH rhythm. Fluphenazine treatment caused a decrease concentration of α-MSH in the brain at all times of the day. In contrast, housing rats in chronic darkness resulted in an increase in α-MSH concentrations at all times of the day. These results indicate a relationship of monoaminergic and α-MSH neurons in the brain.

Significance to Biomedical Research and the Program of the Institute: The present studies demonstrate the presence of α-MSH and deacetylated α-MSH in the rat and human brain and suggest that an acetylation process may selectively regulate the activity of the α-MSH-containing neuron by altering the behavioral activity of the released peptide. The basic chemical, physiological and behavioral results described here lay the groundwork for further studies of the physiology and pathophysiology of α-MSH in the central nervous system.

Proposed Course of Project: Further studies are in progress to determine the transynaptic actions of α-MSH and influence of physiological and psychological processes. α-MSH concentrations will be determined in the CSF of patients with various neurological and psychological disorders to examine the role of this peptide in neuropathology.

Publications:

O'Donohue, T.L., Miller, R.L. and Jacobowitz, D.M.: Identification, characterization and stereotaxic mapping of intraneuronal α-melanocyte stimulating hormone-like immunoreactive peptides in discrete regions of the rat brain. Brain Research 176: 101-123, 1979.

O'Donohue, T.L., Miller, R.L., Pendleton, R.C. and Jacobowitz, D.M.: A diurnal rhythm of immunoreactive α-melanocyte stimulating hormone in discrete regions of the rat brain. Neuroendocrinology, 29: 221-300, 1979.

O'Donohue, T.L., Holmquist, G.E. and Jacobowitz, D.M.: Effect of hypophysectomy on α-melanotropin concentrations in discrete regions of the rat brain. Neuroscience Letters, 14: 271-274, 1979.

O'Donohue, T.L., Charlton, C.G., Helke, C.J., Jacobowitz, D.M., Miller, R.L.: Identification of α-melanocyte stimulating hormone immunoreactivity in rat and human brain and cerebrospinal fluid. In Gross. E. and Meienhofer, J. (Eds.): Peptides, Structure and Biological Function. Rockford, Ill., Pierce Chemical Co., 1979, pp. 897-900.

O'Donohue, T.L. and Jacobowitz, D.M.: Studies of α-MSH-containing nerves in the brain. Neuroactive Compounds and Their Cell Receptors. Basel, Karger Press, 1980, in press.

O'Donohue, T.L., Miller, R.L., Pendleton, R.C. and Jacobowitz, D.M.: Demonstration of an endogenous circadian rhythm of α-melanocyte stimulating hormone in the rat pineal gland. Brain Research 186: 145-155, 1980.

O'Donohue, T.L. and Jacobowitz, D.M.: Studies of α-melanotropin in the rat brain. Polypeptide Hormones, 12th Miles International Symposium. 1980.

O'Donohue, T.L., Massari, V.J., Tizabi, Y. and Jacobowitz, D.M.: Identification and distribution of α-melanotropin in discrete regions of the cat brain. Brain Research Bulletin 4: 829-832, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZOL MH 00398-02

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Correlation of Aggressivity with Neurotransmitters in Discrete Brain Areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	V. John Massari	Department of Pharm.	Howard	Univ.
	Nguyen B. Thoa	Pharmacologist	LCS	NIMH
	Gertrude D. Maengwyn-Davies	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

Office of the Chief
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LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .4	PROFESSIONAL: .3	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

TERMINATED

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00399-02 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Neurochemical and Immunochemical Studies of Peptidergic Pathways of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.4	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

TERMINATED

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00351-06 LCS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	M. A. Elchisak	Guest Worker	LCS NIMH
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	R. J. Polinsky	Staff Physician	LCS NIMH
	R. S. Burns	Guest Worker	LCS NIMH
	C. R. Merril	Sr. Research Scientist	LGCB NIMH
	R. L. Hawks	Chemist	NIDA
	S. P. Markey	Pharmacologist	LCS NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Experimental Therapeutics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to develop techniques for studying central nervous system monoamine metabolism in man, and to carry out pharmacokinetic studies of psychoactive drugs. Studies of the kinetics and clearance of deuterated homovanillic acid (HVA) from the circulation continued in rhesus monkeys and patients. Problems with the application of this technique to study CNS dopamine turnover in man were resolved by further study of the kinetics and volume distribution of the metabolite, HVA, before and after probenecid administration. A GC-MS assay for dopamine sulfate ($DASO_4$) was utilized to study DASO4 as a metabolite of dopamine in the circulation and in CSF. The concentration of conjugated DA in serum is 20 - 30 picomoles/ml. Levels of free dopamine in lateral ventricle CSF are approximately 0.75 picomole/ml, while conjugated DA is 2 - 3 picomoles/ml. $DASO_4$ does not appear to cross the blood-brain barrier. A pharmacokinetic study of phencyclidine (PCP) was carried out in the monkey. The plasma concentration - time curve for PCP is described by a two compartment model with a terminal half-life of 200 minutes. Assay for the 4-OH-piperidine and 4-OH-cyclohexanol metabolites were developed.

Project Description:

Objectives: 1. To develop techniques for studying kinetic parameters of central nervous system monoamine metabolism in man. 2. To carry out pharmacokinetic studies of psychoactive drugs. 3. To utilize these techniques to study patients with a variety of psychiatric, neuropsychiatric, and psychosomatic disorders.

Methods Employed: 1. Biochemical methods: Flourometric, gas chromatographic, and mass spectrometric methods are used for assay of endogenous catecholamine metabolites in tissues and body fluids. Mass spectrometric methods are used for assay of deuterium labelled catecholamine metabolites administered exogenously or synthesized endogenously. Gas chromatographic and radio-immunoassay techniques are used for assay of drugs in body fluids (probenecid and d-amphetamine). 2. Physiological methods: As the project progresses, an increasing number of metabolic experiments will be carried out in patients. However, as new procedures are being developed, appropriate experiments will be carried out in rat brain tissue and in primates using serial sampling of blood, cerebrospinal fluid, and urine as necessary. To facilitate these preclinical in vivo experiments on brain metabolism, a small primate metabolic laboratory has been developed with the capacity to maintain chronic collection of lateral ventricle or lumbar cerebrospinal fluid from chaired rhesus monkeys for days or weeks at a time.

Major Findings: Substantial progress has been made on the development of a method to estimate the rate of brain homovanillic acid (HVA) production by a relatively non-invasive technique utilizing blood samples only. This method uses information about the kinetics of HVA distribution and elimination both before and after administration of probenecid. Probenecid blocks the transport of HVA from the brain and CSF into the blood and also blocks removal of HVA from the blood into the urine. The rate of increase in plasma levels of HVA after probenecid administration reflects the rate of formation of HVA in the peripheral organs. The amount of HVA derived from the brain is then estimated from the difference between the rate of HVA formation before and after probenecid administration.

Studies utilizing this technique have been completed in the Rhesus monkey (Elchisak, Ebert, and Kopin). Rapid intravenous injection of HVA labelled with five deuterium atoms (d_5-HVA) was used to label the peripheral body pool of endogenous HVA (d_0-HVA). Experiments were conducted both with and without probenecid administration. The concentrations of both d_0- and d_5-HVA were then determined in sequential samples of blood serum and urine by gas chromatography-mass spectrometry. The calculated basal rate of HVA production by the whole body, including brain, was 1.0 - 1.4 mg/day. The amount of HVA derived from the brain in these animals was calculated to be 55-63 percent of the total daily production.

Preliminary studies on brain HVA production have been similarly conducted in three human normal volunteers (Elchisak, Polinsky, Ebert, and Kopin). The decline of plasma d_5-HVA concentration with time is biexponential, with a biological half-life ranging from 63 to 79 minutes. During probenecid infusion, the decline of plasma d5-HVA was triexponential in two patients, and biexponential in the third. The biological half-life was 113 - 170 minutes. The calculated basal rate of HVA production was 6.2 - 7.8 mg/day. The amount of HVA derived from the brain in these volunteers was calculated to be 51 - 75 percent of the total daily production.

Dopamine sulfate ($DASO_4$) is a metabolite of dopamine (DA) which occurs in several animal species. Work has continued to demonstrate the occurrence and possible physiological significance of this compound as a metabolic intermediate in primates, including humans (Elchisak and Ebert). Conjugated DA, primarily $DASO_4$, occurs in much greater concentrations than free DA in all body fluids studied thus far. Excretion of free DA in human urine is approximately 2 - 4 μmoles/day, while the excretion of conjugated DA, primarily $DASO_4$, is 4 - 8 μmoles/day. In individual subjects, free DA excretion accounts for about one-third of the total (free plus conjugated) daily DA excretion. The concentration of free DA is approximately 0.5 - 1 picomole/ml in human serum, while conjugated DA is 20 - 30 picomoles/ml. Both $DA\text{-}3\text{-}SO_4$ and $DA\text{-}4\text{-}SO_4$ isomers have been detected in urine from Rhesus monkeys, while only $DA\text{-}3\text{-}SO_4$ has been found in human urine.

Levels of free DA in CSF taken from the lateral ventricle of the Rhesus monkey are approximately 0.75 picomole/ml, while conjugated DA is 2 - 3 picomoles/ml. In lumbar spinal CSF, free DA is approximately 0.25 picomole/ml, and conjugated DA is about 3 picomoles/ml. Preliminary results from intravenous infusion of $^{14}C\text{-}DASO_4$ into Rhesus monkeys suggest that neither $^{14}C\text{-}DA\text{-}3\text{-}SO_4$ nor $^{14}C\text{-}DA\text{-}4\text{-}SO_4$ crosses the blood-brain or blood-CSF barrier in this species. This suggests that the substantial quantities of $DASO_4$ found in the CSF come from the brain or spinal cord, and not from the blood. These same experiments showed that steady-state levels of total radioactivity were approached within four hours after beginning the constant-rate infusion. The biological half-life of $^{14}C\text{-}DASO_4$ is therefore one hour or less in the Rhesus monkey.

A package of computer programs, KRVFIT, has been designed for the analysis and display of pharmacokinetic data (Goering and Elchisak). This package uses the digital computer program MLAB, to analyze pharmacokinetic data by nonlinear regression techniques, but uses another program, ESTINI, to calculate initial parameter estimates. The combination of these programs, together with other programs, results in a relatively simple, yet powerful software package for the analysis of polyexponential pharmacokinetic data.

Pharmacodynamic, pharmacokinetic, and metabolic studies of phencyclidine were carried out in animals in the past year. A rat model of urinary acidification treatment was developed to test the pH-dependence of the excretion of phencyclidine (PCP). NH_4Cl was administered in the drinking water prior to drug administration, resulting in a urinary pH of 5.4 - 5.6. After administration of ^3H-PCP, total radioactivity and unchanged drug were measured.

Urinary acidification resulted in a 2 to 3 fold increase in the excretion of PCP (3.0% vs. 8.1%). The excretion of the hydroxy metabolites was unchanged. The relationship between the pKa, the permeability of the nonionized form, and the metabolic rate is such that no significant change in the net quantity of drug excreted was observed following urinary acidification in the rat.

Subgroups of the general population are susceptible to the development of behavioral effects such as excitement or to a prolonged recovery period following administration of PCP. To investigate these clinical phenomena, the single IV dose plasma kinetics and relationship of plasma levels to neurological effects of PCP and it mono-hydroxy metabolites were studied in the monkey. The GC-MS assay for PCP was adapted from the literature. A GC-MS assay for the 4-OH-piperidine and 4-OH-cyclohexanol metabolites was developed in our laboratory (sensitivity 1 ng/ml). A two compartment model ($T1/2_{\alpha}$ = 27 min., $T1/2_{\beta}$ = 205 min.) best described the concentration-time curve for PCP. A 'deep' compartment related to the neurological effects of PEP in the monkey was not demonstrated with the dosage used and the 1 ng/ml sensitivity of the assay. However, it was shown that the neurological effects of the drug are related to the plasma level. Prolonged recovery may reflect a slow rate of 4-hydroxylation in a subgroup of the population.

Studies of the pharmacological activity of the mono-hydroxy metabolites in the monkey revealed that the 4-OH-piperidine possesses PCP-like activity, but is one-fifth to one-tenth as potent as PCP and induces seizures at a lower dosage. The 4-OH-cyclohexanol produced an agitated excited state in the monkey. Behavioral toxicity in the form of an excited state in man may be related to the kinetics of the 4-OH-cyclohexanol metabolite which is the major metabolite in man (Burns, Hawks, Ebert).

Two new clinical studies of catecholamine metabolism have begun this year. A technique to study epinephrine turnover in man is being developed by using deuterated methionine to label the N-methyl group of epinephrine during its synthesis in the adrenal gland. The technique will be used to study the clinical pharmacology of SKF 64139, a PNMT inhibitor. In collaboration with Dr. Robert Kessler (Nuclear Medicine Department, Clinical Center), preliminary studies are in progress to develop a labelling technique for brain dopamine neurons that would be applicable to positron emission tomography. This effort involves the production of ^{18}F labelled precursors of dopamine or dopamine receptor ligands whose pharmacology is not different from the nonlabelled species.

Significance to Biomedical Research: Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate of amine utilization. The development of more quantitative methods for determining central nervous system amine utilization in man is essential for testing hypotheses regarding the role of amines in psychiatric, medical, and neurological disorders, and assessing the effects of drugs on the amines of the brain. Studies of the kinetics, metabolism, and behavioral effects of PCP bear directly on the psychiatric and neurological consequences of abuse of this drug.

Proposed Course: The metabolic techniques described above for the study of catecholamine metabolism are now being applied to studies in normal volunteers and patient groups.

Development of a GC-MS assay for the di-hydroxy metabolite of PCP, and further development of the mono-hydroxy metabolite assay with the use of a capillary column and negative ion mass spectrometry is planned. Future studies in animals include outlining the 'fall-off' kinetics of PCP following administration of multiple IM doses to steady state to demonstrate a deep compartment. Studies in man of several aspects of the pharmacology of PCP are planned for the coming year. These include the single IV dose plasma kinetics of PCP; individual variation of 4-hydroxylation of PCP; the relationship between the kinetics of the 4-OH-cyclohexanol and the behavioral effects of the drug in man.

Publications:

1. Ziegler, M.G., Lake, C.R., and Ebert, M.H.: Norepinephrine elevations in cerebrospinal fluid after d- and l-amphetamine. European Journal of Pharmacology 57:127-133, 1979.

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3. Ziegler, M.G., Lake, C.R., Wood, J.H., and Ebert, M.H.: Norepinephrine in cerebrospinal fluid: Basic studies, effects of drugs and disease. In, Neurobiology of Cerebrospinal Fluid. New York, Plenum Press, 1980.

4. Ebert, M.H., Kartzinel, R., Cowdry, R.W., and Goodwin, F.K.: Cerebrospinal fluid amine metabolites and the probenecid test. In, Wood, J. (Ed.): Neurobiology of Cerebrospinal Fluid, pp. 97-112, New York, Plenum Press, 1980.

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7. Ebert, M., Elchisak, M., Zavadil, A., Polinsky, R., and Kopin, I.: Use of deuterium-labelled methionine and HVA to study dopamine metabolism in vivo. In, Catecholamines: Basic and Clinical Frontiers, Usdin, E., Kopin, I.J., and Barchas, J. (Eds.), pp. 889-891, Pergamon Press, New York, 1979.

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11. Lerner, P., Major, L.F., Ziegler, M., Dendel, P.S., Ebert, M.H.: Central noradrenergic adaptation to long-term treatment with imipramine in rhesus monkeys. Brain Research, in press.

12. Eisler, T., Teräväinen, H., Nelson, R., Krebs, H., Weise, V., Lake, C.R., Ebert, M.H., Whetzel, N., Murphy, D.L., Kopin, I.J., Calne, D.B.: Deprenyl in Parkson's disease. Neurology, in press.

13. Ebert, M.H.: Techniques for the study of CNS amine metabolites in man. Psychopharmacology Bulletin, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00352-05 LCS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
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	W. H. Kaye	Staff Psychiatrist	LCS NIMH
	H. Weingartner	Acting Chief, LPP	LPP NIMH
	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	C. R. Merril	Sr. Research Scientist	LGCB NIMH
	L. Nee	Clinical Social Worker	LCS NIMH
	N. Sitaram	Research Psychiatrist	BPB NIMH
	J. C. Gillin	Chief, Unit on Sleep Studies	BPB NIMH
	P. Gold	Chief, Unit on Neuroendocrinology	LCP NIMH

COOPERATING UNITS (if any)

Section on Medicine; Laboratory of Psychology and Psychopathology, NIMH; Laboratory of General and Comparative Biochemistry, NIMH; Unit on Sleep Studies

LAB/BRANCH

Laboratory of Clinical Science

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Section on Experimental Therapeutics

INSTITUTE AND LOCATION

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2.5	2.0	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several neuropsychiatric syndromes including Alzheimer's dementia, Gilles de la Tourette's syndrome are being studied in terms of memory function, neuroendocrine function, and possible pharmacological treatment. The pathophysiology and neuropharmacology of anorexia nervosa and orthostatic hypotension is also under investigation. Clinical trials are underway with several cholinergic agents (lecithin, THA, arecholine) with regard to memory function in Alzheimer's dementia. A clinical trial of lecithin in Gilles de la Tourette's syndrome was not efficacious. The blood pressure responses to infusions of norepinephrine, angiotensin, and tyramine has shown that idiopathic orthostatic hypotension patients have alpha adrenoreceptor supersensitivity while those patients with multiple system atrophy, in addition, lack baroreceptor modulation of the pressor response. Insuline tolerance studies have revealed that the autonomic defects in patients with chronic autonomic failure may include vagal dysfunction and deficient adrenal medullary responses to hypoglycemia, however, other counter-regulatory hormones (glucagon, growth hormone, cortisol) are secreted appropriately despite the absence of a catecholamine response. Cerebrospinal fluid studies in anorexia nervosa have demonstrated decreased serotonin and dopamine turnover during the acute illness.

Project Description:

Objectives: The purpose of this study is to investigate several neuropsychiatric syndromes from the perspective of memory and intellectual function, neuroendocrine function, neurotransmitter metabolism, and possible pharmacological treatment. Currently under study are several dementias with known neuropathology, particularly Alzheimer's dementia. Other diseases under intensive study are Gilles de la Tourette's syndrome, anorexia nervosa, and orthostatic hypotension.

Methods Employed: Methods for studying information processing in neuropsychiatric disorders include learning tasks that permit the assessment of stimulus and modality specific information in processing (1) short term memory; (2) long term memory; (3) retrieval processing; (4) encoding; (5) the interrelationships of these processes; and (6) factors that might influence decay and interference of memory in these systems. Procedures are used to test free recall, serial learning, cued recall, and recognition memory.

One strategy for identifying neurotransmitter systems that may play a role in the pathogenesis of psychiatric or neurological symptoms is to study the clinical effects of a series of drugs having relatively specific stimulating or blocking effects on a particular neurotransmitter. This approach has been profitably applied to research on affective disorders. We are pursuing a pilot investigation of this nature in patients with dementias of varying etiologies, focusing on the adrenergic and cholinergic systems.

Methods used in neuroendocrine protocols are established techniques of studying plasma prolactin and growth hormone responses to the acute administration of drugs known to affect dopaminergic neurotransmission. From these endocrine responses one can infer the functional state of dopaminergic transmission in the tuberoinfundibular systems. Methods used in Stage II drug trials are established methods for the evaluation of psychotropic drugs including double-blind design, appropriate rating scales, placebo controls. Methods used in studies of neurotransmitter metabolism are those methods outlined in Project Z01 MH 00351-06 LCS.

Major Findings: Neuropharmacological assessment of autonomic dysfunction has continued in patients with chronic autonomic failure (Polinsky, Ebert, Kopin). A longitudinal study of plasma catecholamines and pressor responses is in progress. Analysis of the glucose recovery curves following insulin-induced hypoglycemia in patients with orthostatic hypotension (Polinsky, Kopin, Ebert, Weise) shows that there is a mild insulin resistance in those patients with absent adrenal medullary responses. Basal cortisol levels are elevated in patients with adrenergic insufficiency; the sequence, magnitude, and temporal profile of glucagon, growth hormone, and cortisol are unaltered despite the absent catecholamine response to hypoglycemia in these patients. Pancreatic peptide has been shown to correlate with vagal activity in both animals and man; plasma levels of this peptide are increased in response to both hypoglycemia and following a meal. Plasma pancreatic peptide levels were measured (in collaboration with Dr. Ian Taylor, UCLA) during insulin-induced hypoglycemia in orthostatic hypotension patients as well as normal controls. In normals, pancreatic peptide levels in plasma reached a maximum at 50 minutes after administration of insulin. Idiopathic orthostatic hypotension patients showed no elevations in their plasma pancreatic peptide levels; patients with multiple system atrophy showed variable pancreatic peptide responses. There was no correlation between catecholamine levels and the response of this vagal activity marker. Patients have been identified with absent catecholamine responses accompanied by intact pancreatic peptide activity and also vice versa. Another hormone which has been measured in response to hypoglycemia is gastrin (in collaboration with Dr. Ian Taylor, UCLA). Patients with idiopathic orthostatic hypotension have elevated fasting levels of gastrin with a normal increment following hypoglycemia. Multiple system atrophy patients have normal fasting gastrin levels but manifest a reduced increment in response to hypoglycemia. Two individuals had extremely high fasting gastrin levels suggesting a more complete denervation of the neural mechanism controlling gastrin function. A study of gastrointestinal hormonal responses to a standard test meal is in progress. Initial results show that blood pressure drops precipitously within minutes after oral intake in orthostatic hypotension patients. There does not appear to be a significant change in plasma catecholamines during eating in normals. This preliminary data has prompted the search for a vasodilator substance which might be released during feeding.

Examination of CSF levels of norepinephrine (Polinsky, Lake, Ebert) has shown that those patients with idiopathic orthostatic hypotension have low CSF norepinephrine compared to controls while the CSF level in patients with multiple system atrophy is either normal or elevated. A study of other neurotransmitters and peptides in the CSF is in progress. The neural control of melatonin rhythm is exerted through the beta sympathetic nervous system. Measurement of 6-hydroxymelatonin (in collaboration with the Section on Mass Spectroscopy) in the urine reveals that some patients with orthostatic hypotension have very low levels of melatonin while other patients have a normal amount but altered diurnal pattern of melatonin secretion. Analysis of data is still in progress.

Measurement of ligand binding to alpha receptors of red blood cells and platelets is thought to parallel changes which occur at the vascular alpha receptor. Preliminary analysis of this data (in collaboration with Dr. Kafka, APB) suggests that male Shy-Drager syndrome patients and female idiopathic orthostatic hypotension patients have increased alpha receptor sites. Correlation of these results with plasma catecholamines and pressor responses is in progress.

Sensitivity of skin fibroblast and lymphoblast cultures to various forms of radiation have been shown to correlate with primary neuronal degeneration in a number of inherited neurological illnesses. IOH and MSA involve nerve cell death in the central nervous system; a study of the radiation sensitivity of cell cultures from these patients has been initiated (in collaboration with Dr. J. Robbins, NCI). A similar investigation is in progress with Huntington's disease and their at-risk relatives.

Clinical evaluation of orthostatic hypotension patients has shown that the blood volume and RBC mass, as measured by Cr^{51} - RBC labelling, is reduced in about two-thirds of the patients (in collaboration with Dr. L. Corash, Hematology Service). Hematologic studies including serum folate, vitamin B^{12}, iron levels, ferritin and bone marrow are normal. Assay of serum 2,3 - DPG and erythropoietin levels are in progress as well as an attempt to correlate the anemia with changes in renal function.

A number of treatment protocols for orthostatic hypotension have been initiated. Medications which block prostaglandin synthesis have been most efficacious. The mechanism of action is not entirely clear and hence, measurement of urinary protaglandins, plasma catecholamines, renin and pressor sensitivity to norepinephrine are currently being investigated before and after treatment. Other medications which are currently bing tested include mianserin, midodrine, propranolol, and fludrocortisone.

In Gilles de la Tourette's syndrome (TS), a therapeutic trial of lecithin (Polinsky, Ebert, Caine) has been completed in an attempt to augment the cholinergic side of the striatal dopamine-acetylcholine imbalance which has been postulated as a cause in this disorder. Although there were some minor improvements in either motor or vocal tics in individuals, the overall group responses showed no difference compared to the placebo treatment. Neuological examination of 48 patients with TS revealed that 57% exhibit "soft neurological signs." Studies of obstetric, birth, and developmental records, family background and speech and language studies are still in progress. Since a greater number of adult TS patients have been seen in the outpatient clinic, it is possible to embark on sleep and CSF studies which have not been possible in the younger population due to the difficulty in obtaining control subjects. Haloperidol remains the treatment of choice for all patients requiring medication in our outpatient clinical population.

Pharmacological studies of memory function in Alzheimer's disease have continued this year in collaboration with Drs. Weingartner, Sitaram, and Gillin. Various parameters of memory processing and retrieval are being documented by Dr. Weingartner. Several pharmacological approaches to modifying brain cholinergic function have been studied. Infusion of arecholine (a cholinergic receptor agonist) produces transient improvement in serial learning tasks. A trial of lecithin (a dietary source of choline) and THA (an oral cholinesterase inhibitor) used in combination produced a significant improvement in memory function in five of nine patients studied.

A clinical genetic study of familial Alzheimer's disease is nearing completion (Ms. Nee, Drs. Polinsky, Ebert). A single family has been studied, and the pedigree has the following characteristics: 9 generations, 821 identified individuals, 34 individuals with a presumptive diagnosis of Alzheimer's disease, 2 individuals with a neuropathological diagnosis, 9 individuals living with a clinical diagnosis, 54 individuals evaluated at the NIMH, 111 individuals from whom blood samples were obtained. A formal genetic analysis of HLA antigens is in progress in collaboration with Dr. Weitkamp, University of Rochester.

A clinical research program in primary anorexia nervosa has continued (Drs. Kaye, Ebert, Lake). A study of central nervous system dopamine, serotonin, and norepinephrine metabolism was completed using CSF levels of amines and amine metabolites. Four groups were compared: anorexia nervosa patients at minimal weight, the same patients immediately after weight gain, "recovered" anorexia patients at normal weight for six months but with continued psychiatric symptoms, and normal female controls. At minimal weight a 40% decrease in HVA and a 20% decrease in 5HIAA was observed compared to the other three groups which did not differ from each other. This indicates a decrease in dopamine and serotonin turnover in the acutely starved phase of anorexia nervosa. In contrast, CSF norepinephrine levels are significantly lower in the group of "recovered" anorexia patients compared to the other groups. In collaboration with Dr. Gold (CPB, NIMH) and Dr. Robertson (U. of Chicago), vasopressin (AVP) function was studied. Anorexia nervosa patients have an erratic secretory response of AVP to a hypertonic saline infusion compared to the linear change in AVP to increasing plasma osmolality in normals. Anorexia nervosa patients have a high CSF/plasma ratio of AVP compared to normals. This CSF AVP abnormality is most extreme in the "recovered" group above, and CSF AVP levels have a reciprocal relationship to CSF NE levels. These neurochemical data provide additional evidence of an abnormality in hypothalamic function in these patients, and suggest that the abnormality persists after immediate weight gain. Norepinephrine and AVP have both been implicated in the regulatory mechanisms of appetite in animals.

Collaborative projects continue with the Childhood Mental Illness Unit of the BPB, NIMH, on the clinical pharmacology of stimulants, L-dopa, and tricyclic antidepressants in the treatment of hyperactive children. Collaborative research continues with Dr. Merril, LGCB, NIMH on clinical applications of two dimensional polyacrilamide gel electrophoresis of proteins, particularly in the study of central nervous system genetic diseases. A summary of these projects is found in project reports Z01 MH 00100-05 BP and Z01 MH 00935-13 LGCB.

Significance of Biomedical Research: In recent years, interest has increased in the biochemical aspects of learning and memory. Neuropharmacological aspects of memory function have also become a focus of interest. Most of the published studies in this area are in animals. However, there is a beginning literature of clinical neuropharmacological investigations of memory, including work on stages of memory storage pursued recently in our program by Drs. Weingartner and Murphy. We hope to extend knowledge about pharmacological and biochemical aspects of memory function by studying several diseases in which disordered memory is a primary symptom, and in which there is existing knowledge about anatomical and biochemical pathology. Gilles de la Tourette's syndrome is a neuropsychiatric syndrome whose pathophysiology is poorly understood. The symptomatology appears to be responsive to drugs which block dopamine receptors. By conducting new drug trials, and gathering data on cognitive and linguistic function, monoamine metabolism, and neuroendocrine function in this illness, we hope to expand knowledge about its pathophysiology and its possible relationship to other psychiatric illnesses such as schizophrenia. Anorexia nervosa is another neuropsychiatric syndrome in which there is little published information concerning pharmacological treatment and neurotransmitter metabolism. It is clearly established in the animal literature that monoamine systems in the hypothalamus play an important role in the normal regulation of appetite. Orthostatic hypotension is a clinical syndrome that can now be divided into subtypes that have etiological and therapeutic implications using new techniques for studying catecholamine metabolism and physiology.

Proposed Course: In the next year the initial phase of cholinergic drug trials in Alzheimer's dementia will be concluded, as well as a variety of neurochemical studies. The results of these studies will indicated whether or not a chronic drug trial is indicated. In Gilles de la Tourette's syndrome we intend to pursue neurochemical studies such as those indicated in project report Z01 MH 00351-06. In anorexia nervosa we plan to pursue further studies of the abnormalities in vasopressin function and central nervous system norepinephrine metabolism outlined above. The orthostatic hypotension project will continue several therapeutic trials begun this year, and continue studies of sympathetic nervous system function and metabolism.

Publications:

1. Merril, C.R., Leavitt, J., Van Keuren, M.L., Ebert, M.H., Caine, E.D.: Hypoxanthine quanine phosphoribusyltransferase (HGPRT) in Gilles de la Tourette's syndrome. Neurology 29:131-134, 1979.

2. Weingartner, H., Caine, E.D., and Ebert, M.H.: Imagery, encoding, and retrieval of information from memory: Some specific encoding-retrieval changes in Huntington's disease. Journal of Abnormal Psychology 88: 52-58, 1979.

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7. Mendelson, W.B., Caine, E.D., Goyer, P., Ebert, M., Gillin, J.C.: Sleep in Gilles de la Tourette's syndrome. Biological Psychiatry 15(2):339-343, 1980.

8. Caine, E.D., Mendelson, W.B., Loriaux, D.L.: Neuroendocrine effects of haloperidol in an adolescent with Gilles de la Tourette's disease and delayed onset of puberty. Journal of Nervous and Mental Disease 167(8):504-507, 1979.

9. Caine, E.D., Polinsky, R.J.: Haloperidol-induced dysphoria in patients with Tourette's syndrome. American Journal of Psychiatry 136:1216-1217, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00271-11 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Measurement of Brain Contribution to Norepinephrine Metabolites in Human Urine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
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	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Robert L. Sherman	Chemist	LCS NIMH
	Peter Blombery	Guest Worker	LCS NIMH
	Jerry A. Oliver	Chemist	LCS NIMH
	Sanford P. Markey	Pharmacologist	LCS NIMH

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Laboratory of Clinical Science

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gas liquid chromatography-Mass Spectrometry (GLC-MS), ion exchange chromatography, high pressure liquid chromatography (HPLC) and other separation procedures are used to isolate and measure the specific activities of deuterium-labelled or ^{14}C-norepinephrine (NE) metabolites, 3-methoxy-4-hydroxyphenylglycol (MHPG) and vanillyl mandelic acid (VMA) in the plasma and urine. D,ℓMHPG-D_3 and D(-)MHPG-D_3 have been injected into human controls and the kinetics of conversion to VMA-D_3 studied in blood and urine. Similar studies are planned in patients with orthostatic hypotension as well as with various neurologic and mental disorders.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

In humans, NE from the sympathetic nervous system and brain and epinephrine from the adrenal medulla are converted to VMA and MHPG. Urinary VMA is 2 to 3 times as great as MHPG. Small amounts (1-5%) of the MHPG is in the free form; the rest is conjugated as the glucuronide or sulfate, in approximately equal quantities. In brain, MHPG is the main metabolite of NE, with very small amounts of VMA being produced. Previous work in this and other laboratories suggested that MHPG in the free form is removed from brain directly to the blood in humans, but is conjugated in brain in several other species.

When ^{14}C dopamine is infused peripherally it does not enter the brain. About 25% is converted to ^{14}C NE and its metabolites. Since almost all VMA is formed peripherally its specific activity (ratio of radioactive to non-radioactive VMA) reflects that of metabolites of peripheral NE. The specific activity of MHPG represents peripheral plus brain NE metabolism. The ratio in the specific activities of VMA and free MHPG can be used to measure the relative amounts of MHPG produced by brain and periphery. The measurement of absolute quantities of MHPG and VMA formed makes it possible to calculate the amount of MHPG produced by brains of control subjects or patients. The specific activities of the glucuronide or sulfate of MHPG relative to that of free MHPG may be an indication of MHPG conjugated in tissues before reaching the circulation.

Since GLC-MS methods are already in use in the Laboratory, O^{18} dopamine was prepared. Work will continue using O^{18} since much less work and time will be required for purification of metabolites.

Methods have been developed for the analysis of plasma for MHPG and VMA using GLC-MS. D,ℓMHPG-D_3 and D(-)MHPG-D_3 have been injected into monkeys and human controls to study the amount of conversion to VMA. Plasma and urine have been analyzed. It has been found that MHPG is rapidly converted to glucuronide and sulfate but a substantial portion is converted to VMA. VMA formed from MHPG accounts for approximately 50% of VMA found in urine.

Results of the dopamine infusion studies are being reassessed taking these results into consideration.

PUBLICATIONS:

Next year this project will become incorporated into Project Z01 MH 00275 on "Release and Turnover of Catecholamine Metabolites in Human Subjects" and will be terminated as a separate entity.

Blombery, P.A., Kopin, I.J., Gordon, E.K., Markey, S.P. and Ebert, M.H.: Conversion of MHPG to VMA and implications regarding significance of urinary MHPG. Arch. Gen. Psych. in press.

Kopin, I.J.: Assessing Norepinephrine Metabolism in Human Brain: Past, Present and Future. In Symposium Proceedings on Psychiatry and the Biology of The Human Brain, Elsevier North Holland, N.Y. 1980. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00272-09 LCS

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Metabolism, Distribution and Biochemical Effects of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Anthony P. Zavadil	Guest Worker	LCS NIMH
	Richard L. Hawks	Guest Worker	LCS NIMH
	Richard S. Burns	Clinical Associate	LCS NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Irwin J. Kopin	Chief, LCS	LCS NIMH

COOPERATING UNITS (if any)
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TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the biochemical effects of drugs in animals and man using mass spectral methodology are being pursued in order to relate pharmacological action to molecular events and clinical efficacy. Tricyclic antidepressants are being quantitated from patients by mass spectral measurements in order to establish therapeutic and predictive indices. Pharmacokinetic studies of phencyclidine and its metabolites in monkeys are in progress.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Pharmacokinetic and metabolism studies of phencyclidine in man are continuing. In the past year, sensitive gas chromatographic-mass spectrometric assays for phencyclidine and its 4-hydroxy cyclohexanol and 4-hydroxy-piperidine metabolites have been developed and applied to primate pharmacokinetic studies. Unusual side effects or toxicity of phencyclidine are seen in animals like man which form mono-hydroxy metabolites. Recent studies in our laboratory have shown that the 4-hydroxy cyclohexanol, the major human metabolite, is active in producing an excited, agitated state in the monkey. Most of the unique effects of phencyclidine are related to plasma concentration in our studies, suggesting that plasma levels in humans should provide valuable clinical end points for monitoring the recovery of overdose patients, predicting recovery time, and making disposition decisions. Furthermore, these studies have indicated that there is no significant change in the quantity excreted following urinary audification in the primate, contrary to the treatment being aggressively pursued in several medical centers. In the coming year, it is planned to study the pharmacokinetics of phencyclidine in human volunteers with a prior history of occasional use. A protocol is being submitted to the Clinical Research Committee to initiate these studies.

A study characterizing the plasma level vs. effect of diphenyl barbituric acid in order to establish an effective anticonvulsant barbiturate derivative lacking the sedative properties of phenobarb has been initiated. A gc assay has been developed which will be applied to animal model (cat) samples to determine whether variability in animal response may be due to the poor solubility of the drug and, therefore, erratic absorption and distribution. Subsequent studies will concentrate on establishing a consistent drug delivery system.

PUBLICATIONS:

Markey, S.P., Colburn, R.W., Kopin, I.J., and Aamodt, R.L.: Distribution and excretion in the rat and monkey of ^{82}Br-bromocriptine. J. Pharmacol. Exp. Ther. 211: 31-35, 1979.

Davis, G.C., Williams, A.C., Markey, S.P., Ebert, M.H., Caine, E.D., Reichert, C.M., and Kopin, I.J.: Chronic Parkinsonism secondary to intravenous injection of meperidine analogues, Psychiatry Res. 1: 249-254, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00274-06 LCS

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Methods of Ionization in Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Leonid Kellner	Visiting Scientist	BEIB DRS

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS

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Laboratory of Clinical Science

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alternate ion sources for enhanced sensitivity and performance of a quadrupole mass analyzer have been constructed and tested in both chemical and electron ionization modes.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Previous success with the modification and application of a mass spectrometer for negative chemical ionization analysis has led to efforts to construct and test alternate ion sources which may have utility in biomedical research. An ion source built within a quadrupole rod assembly was designed, constructed and tested. Preselection of mass was possible with enhanced sensitivity realized in comparison of applied rf field contrasted to its absence. However, arcing precluded the use of this assembly at chemical ionization pressures, and the relatively closed design significantly degraded its sensitivity in comparison to a conventional e.i. source.

Future experiments with an analytical Quistor ion trap are planned.

PUBLICATIONS:

Barden, L.K., Tate, R.L., Schultz, A.R., Markey, S.P., and Oliver, J.A.: Selected ion recording system. Proc. 27th Ann. Conf. on Mass Spectrometry and Allied Topics, 253-254, 1979.

Blombery, P.A., Markey, S.P., and Kopin, I.J.: Negative EC-CI assays for norepinephrine metabolites. Use of hexafluoroacetone to produce cyclic derivatives. Proc. 27th Ann. Conf. on Mass Spectrometry and Allied Topics, 729-730, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00275-03 LCS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Release and Turnover of Catecholamine Metabolites in Human Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Blombery	Guest Worker	LCS NIMH
OTHER:	Irwin J. Kopin	Chief, LCS	LCS NIMH
	Edna K. Gordon	Chemist	LCS NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Sanford P. Markey	Pharmacologist	LCS NIMH
	Jerry A. Oliver	Chemist	LCS NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New methods have been developed using negative ion electron capture chemical ionization gas chromatography-mass spectrometry (EC/CI GC-MS) for the assay of the norepinephrine (NE) metabolites, 3-methoxy-4-hydroxy phenylglycol (MHPG), dihydroxyphenylglycol (DHPG), normetanephrine (NMN) and metanephrine (MN). These methods increase the sensitivity of detection of these metabolites 50-100 fold over available electron impact (EI) GC-MS methods permitting the study of NE metabolism in much smaller amounts of biological material than was previously possible.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Assay of catecholamine metabolites in tissue, blood and urine provides information about neurotransmitter release and pathways of metabolism in different tissues. GC-MS methods of assay, in combination with stable isotope labelling of precursors and their metabolites extends the versatility of this technique by providing assessment of rates of metabolism and clearance. Currently available EI GC-MS methods of assay for NE metabolites are limited in sensitivity, and with more sensitive assays, the above techniques could be extended to study NE metabolism in discrete parts of brain and other tissues, as well as allowing assay in very small volumes of plasma.

In this study, suitable derivatives of NE metabolites were investigated for assay by EC/CI GC-MS. The utilization of EC/CI GC-MS depends upon the formation of stable electron-capturing derivatives which provide a high yield of compound-specific anions under CI conditions.

By using hexafluoroacetone to derivatize MHPG, DHPG, NMN and MN, a stable cyclic ketal was formed with good electron-capturing properties. Further reaction with trifluoroacetic anhydride or butylboronic acid formed derivatives which allowed detection of as little as 1-5 pg of the above metabolites in the EC/CI GC-MS mode. This represents a 50-100 fold increase in sensitivity over currently available methods.

Human plasma contains 5 ng/ml free MHPG, 1 ng/ml free DHPG, and 250 pg/ml free NMN and MN. These are being utilized to observe changes in plasma metabolite levels during sympathetic activation in normal subjects as well as in patients with orthostatic hypotension, hypertension, depression and other psychiatric disorders.

PUBLICATIONS:

Blombery, P.A., Markey, S.P. and Kopin, I.J.: Negative EC-CI assays for norepinephrine metabolites. Use of hexafluoroacetone to produce cyclic derivatives. Proc. 27th Ann. Conf. on Mass Spectrometry and Allied Topics, Seattle, 1979, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00276-01 LCS

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Metabolism of Melatonin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Robert W. Colburn	Chemist	LCS NIMH
	Masahiro Tetsuo	Visiting Fellow	LCS NIMH
	Alfred J. Lewy	Staff Psychiatrist	CPB NIMH
	Ronald Polinsky	Staff Physician	LCS NIMH
	Merrily A. Poth	Clinical Associate	IRP CH
	Irwin J. Kopin	Chief, LCS	LCS NIMH

COOPERATING UNITS (if any)
Sections on Medicine and Experimental Therapeutics, LCS; Clinical Psychobiology Branch, NIMH; IRP, CH

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TOTAL MANYEARS: 3.5	PROFESSIONAL: 3	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The pineal hormone melatonin and its major urinary metabolite 6-hydroxymelatonin are being quantified by gas chromatography-negative chemical ionization mass spectrometry. Plasma melatonin levels in humans have been assayed and reveal that nighttime melatonin release is inhibited by light of daylight intensity. Urinary melatonin metabolite assays have indicated the normal range for daily adult excretion as well as abnormal patterns in patients with diminished adrenergic activity. Studies are in progress to determine the pattern of excretion during normal human growth and development, particularly with regard to puberty and the menstrual cycle. Animal studies are being pursued to determine the contribution of extra-pineal sites of melatonin synthesis to urinary 6-hydroxymelatonin.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

As outlined in projects Z01 MH 00450-04 (CPB) and Z01 MH 01851-02 (CPB), melatonin plasma levels in normal volunteers subjected to various lighting conditions have been determined by gc-negative c.i.m.s., demonstrating that the human pineal gland is responsive to light of the intensity of daylight.

A quantitative assay for urinary 6-hydroxymelatonin sulfate has been developed. Previous efforts by others had lacked the sensitivity and specificity necessary to quantify normal urinary levels. We have utilized a deuterated internal standard which was prepared by feeding deuterated melatonin to rats and isolating 6-hydroxymelatonin sulfate by paper chromatography. After addition of the standard, 3 ml of urine is subject to enzymatic hydrolysis, methylene chloride extraction, derivative formation, and silicic acid column chromatography. The purified extract was analyzed by electron capture negative c.i. using packed column gc and selected ion recording. In a preliminary study, urine was collected from 7 normal adult males at 6 hour intervals for three continuous days. Daily excretion of urinary 6-hydroxymelatonin ranged from 5-15 μg, ranging from a low of 0.14 μg/6 hr during the day to 10.84 μg/6 hr at night.

Studies on the effect of diminished β-adrenergic activity, such as that found in patients with orthostatic hypotension have been initiated. Patients with significant peripheral sympathetic deficits exhibit low plasma norepinephrine levels and an inability to respond to mild stress, such as standing. In lower animals, the pineal is known to be noradrenergically innervated. Patient urines are being analyzed, and, thus far, indicate a markedly diminished excretion of 6-hydroxymelatonin. Patients with central as well as peripheral involvement appear to exhibit a lack of diurnal rhythm or an uncoupled rhythm.

The role of melatonin in some species is antigonadatrophic, suggesting that it may have an important role in human reproductive biochemistry. Urinary metabolite studies in children at various stages of puberty are being initiated in order to test this hypothesis.

Extrapineal sites of melatonin synthesis have been postulated by several laboratories. We have shown that pinealectomy abolishes the plasma melatonin rhythm in the rat, as well as the increased synthesis and release produced by β-adrenergic stimulation. Urinary 6-hydroxymelatonin is being measured in pinealectomized and intact monkeys in order to assess extrapineal synthetic contribution to this metabolite. Light responsivity of monkeys has already been demonstrated, so that these experiments will determine whether there is any significant contribution to the urinary metabolite which is extrapineal and/or light responsive.

PUBLICATIONS:

Sisak, M.E., Markey, S.P., Colburn, R.W., Zavadil, A.P. and Kopin, I.J.: Identification of 6-hydroxymelatonin in normal human urine by gas chromatography-mass spectrometry. Life Sci. 25: 803-806, 1979.

Lewy, A.J., Tetsuo, M., Markey, S.P., Goodwin, F.K., and Kopin, I.J.: Pinealectomy abolishes plasma melatonin in the rat. J. Clin. Endocrinol. Metab. 50: 204-205, 1979.

Tetsuo, M., Markey, S.P., and Kopin, I.J.: Measurement of 6-Hydroxymelatonin in human urine and its diurnal variations. Life Sci. (in press), 1980.

Tetsuo, M. and Markey, S.P.: Quantification of 6-hydroxymelatonin by gc-negative cims. Proc. 28th Ann. Conf. Mass Spectrometry and Allied Topics (in press),1980

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00277-01 LCS

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Synthesis of Stable Isotope Labelled Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Wilfried J.W. Mayer	Visiting Fellow	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Syntheses of oxygen-18 and deuterium labelled compounds are being developed for clinical studies on their metabolic fate and for internal standards in mass spectral assays. General synthetic reactions are being studied in an effort to provide key labelled intermediates in high yield. Syntheses of deuteromethylated catecholamine metabolites have been completed. Optically resolved 4-hydroxy-3-deuteromethoxyphenylethyl glycol (CD_3-MHPG) has been prepared for a clinical study. Oxygen-18 labelled dopamine has been prepared by acid catalyzed exchange. Preparation of oxygen-18 substituted catechols has been achieved in high yield through chromium tricarbonyl complexed dihalobenzene intermediates.

PROJECT DESCRIPTION:

Stable isotope analogs or isotopimers of biogenic amines are being used for human clinical research (Z01 MH 00275-03 LCS) to determine the metabolism and kinetics of distribution and excretion of neurotransmitters. Common to many of the compounds of interest are catechol oxygens, generally enzymatically monomethylated during metabolism. Thus, routes of synthesis of oxygen-18 labelled catechols or mono-deuteromethylated catechols are being pursued.

Optically resolved D(-) CD_3MHPG (200 mg) was prepared using a synthetic sequence we previously reported, with fractional crystallization of the precursor CD_3-4-hydroxy-3-methoxy mandelic acid as its brucine diastereomeric salt. The compound was shown to exhibit the same rotation as the natural norepinephrine urinary metabolite, and has been employed in a kinetic and metabolic study in humans (Z01 MH 00275-03 LCS).

Routes for the synthesis of dopamine-$^{18}O_2$ were explored, principally via production of $^{18}O_2$-catechol from dichlorobenzene. In the course of these efforts it was learned that certain catechols can be directly exchanged with $H_2{}^{18}O$ in an acid catalyzed process. This method has been used to prepare 100 mg of dopamine-$^{18}O_2$ (84.4% $^{18}O_2$, 14.7% $^{18}O_1$).

Chromium tricarbonyl complexes of halogen substituted arenes undergo nucleophilic replacement by alkoxide ions at considerably enhanced rates with respect to the free arenes. The complexes of o-difluoro- and o-dichlorobenzene were prepared and reacted with $CH_3{}^{18}OH$ and base at room temperature. The o-difluorobenzene complex reacts rapidly to yield veratrole-$^{18}O_2$ in yields $\geq$ 90%. Future synthetic effort will focus on the conversion of substituted catechols to 3,4 dihydroxy-$^{18}O_2$-phenylethylglycol and related compounds.

PUBLICATIONS:

Markey, S.P., Powers, K., Dubinsky, K., and Kopin, I.J.: General methods for the synthesis of methyl isotope labelled catecholamine metabolites. Preparation of 4-hydroxy-3-methoxy-d_3(mandelic acid, phenylacetic acid, and phenylethylene glycol). J. Labelled Compd. Radiopharm. 17: 103-113, 1980.

Markey, S.P., Mayer, W.J.W., and Oliver, J.: Synthesis of stable isotope analogs of biogenic amines. Proc. 28th Ann. Conf. Mass Spectrometry and Allied Topics (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00424-05 LCS

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biological Active Peptides in the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI's:	M. Brownstein	D.K. Meyer	LCS NIMH
	R. Eskay	L.E. Eiden	LCS NIMH
	M.C. Beinfeld	J. Furness	LCS NIMH
Other:	H. Gainer, J. Russell, Y.P. Loh		LDN NICHHD
	J. Roth		IRP NIAMDD
	S. Young, M. Kuhar		Hopkins
	E. Schiffman		LDBA NIDR
	C. Bennett		Merck Co.
	J. Kebabian		NINCDS
	J. Schaeffer		U.Cal. S.D.
	S. Hays, S. Paul		CP NIMH
	R. Jensen		NIAMDD
	J. Phillips		U. Iowa
	C. Latker		LVR NEI

COOPERATING UNITS (if any)
LDN NICHHD, IRP NIAMDD, Hopkins, LDBA NIDR, Merck Co., NINCDS, U. Cal. S.D., CP NIMH, NIAMDD, U. Iowa, LVR NEI

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.75		0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued to study the distribution of peptide-containing cells in the central nervous system, the biosynthesis of biologically active peptides, and the factors that regulate peptide secretion. Our studies of a number of peptides have contributed to a better understanding of the cell biology of peptidergic neurons and of their role in the brain.

PROJECT DESCRIPTION:

Objectives: To locate specific groups of neurons in the central and peripheral nervous systems which synthesize and secrete peptides; to determine the biochemical mechanisms underlying the synthesis of peptides by neurosecretory cells; to study interactions between peptidergic and non-peptidergic neurons.

Methods Employed: Biosynthetic, enzymatic and histologic procedures; microdissection techniques; immunocytochemistry.

Major Findings: The members of the Unit on Neuroendocrinology have continued to study biologically active peptides. The peptides are present in central and peripheral nerve cells; these neurons must be located and characterized. In addition, information is needed about the synthesis, axonal transport, and release of the peptides and about the regulation of these processes. This information is essential if we are to understand the roles of biologically active peptides in the brain and periphery.

Studies on the biosynthesis of vasopressin, oxytocin, and neurophysin (Np) have continued. The vasopressin/Np and oxytocin/Np precursors have been described and a third Np precursor has been discovered. In the course of this work a glycopeptide that probably constitutes part of the N-terminus of the vasopressin/Np precursor has been isolated. Its structure is being determined in collaboration with Dr. C. Bennett.

An antichemotactic factor from tumor cells is in the process of being purified and characterized (Drs. Brownstein and Schiffman). This factor may enable the tumor to protect itself from attack by the host.

The distribution of insulin receptors in rat brain has been examined by a new autoradiographic method (Drs. Young, Kuhar, Roth, Brownstein). The density of receptors is especially high in fetal rats suggesting a role for, insulin or a related molecule in CNS growth or development.

The details of regulation of α-MSH secretion from the intermediate lobe of the pituitary by catecholamines has been described (Drs. Eskay and Kebabian). The structures of the α-MSH-like peptides in brain have investigated. Desacetyl α-MSH has been demonstrated in brain extracts (Drs. Loh and Eskay).

A number of neuropeptides have been found in mammalian and amphibian retinae and, more recently, immunocytochemistry has been used to localize these peptides to specific cells (Dr. Eskay). Along similar lines, methods for dissociating and segregating retinal neurons have been developed (Drs. Schaeffer and Brownstein), and now large numbers of photoreceptor and ganglion cells can be prepared for biochemical studies.

The distribution of cholecystokinin (CCK) in the brain has been described and the immunologically active material in brain extracts characterized by high performance liquid chromatography (Drs. Beinfeld and Meyer). The studies have focused on the hypothalamo-neurohypophysial system and have shown that CCK is present in the posterior pituitary and released in parallel with oxytocin and vasopressin. A specific, high affinity binding site for CCK has been demonstrated to be present on brain membranes (Drs. Hays, Paul, Jensen, Beinfeld), and studies of distribution and regulation of receptors are under way.

The innervation of the supraoptic nucleus has been examined (Dr. Meyer) and a natriuretic factor partially characterized. In a related study, the angiotensin-like material in gut and brain is being characterized (Drs. Furness, Phillips, Meyer).

Partial characterization of the LHRH-like peptide in frog sympathetic ganglion has been accomplished (Dr. Eiden). This peptide has received a great deal of attention from electrophysiologists; it is not identical to LHRH. Dr. Eiden is currently engaged in studying the regulation of enkephalin biosynthesis and release in cultured adrenal chromaffin cells.

The retinal toxicity of methylazoxymethanol (MAM), which has been found to produce a fairly unique retinal lesion in fetal and neonatal rats, is being studied by Drs. Eiden, Latker, and Zatz.

In a series of immunocytochemical studies in the gut, several peptides (e.g., somatostatin, bombesin) have been localized to specific neuronal groups. The immunoreactive species have been characterized by high performance liquid chromatography (Dr. Furness).

Significance to Biomedical Research: Peptides secreted by neurons in the CNS appear to act not only as factors which control the anterior pituitary but also as agents (neurotransmitters) which allow nerve cells to interact with one another. Thus it is important to develop methods for studying these compounds.

Proposed Course of Project: The effect of endocrinological surgical and pharmacological manipulations on peptide synthesis and secretion will be studied.

PUBLICATIONS:

Brownstein, M.J., Russell, J.T., and Gainer, H.: Synthesis, transport, and release of posterior pituitary hormones. Science 207: 373-378, 1980.

Meyer, D.K. and Brownstein, M.J.: Effect of surgical deafferentation of the supraoptic nucleus on its choline acetyltransferase content. Brain Res., in press, 1980.

Eskay, R.L., Long, R.T., and Iuvone, P.M.: Evidence that TRH, somatostatin and substance P are present in neurosecretory elements of the vertebrate retina. Brain Res., in press, 1980.

Meyer, D.K., Oertel, W., and Brownstein, M.J.: Deafferentation studies on the glutamic acid decarboxylase content of the supraoptic nucleus of the rat. Brain Res., in press, 1980.

Beinfeld, M.C., Jensen, R.T., and Brownstein, M.J.: HPLC separation of cholecystokinin peptides--two systems. Journal of Liquid Chromatography, in press, 1980.

Eiden, L.E. and Eskay, R.L.: Characterization of LRF-like immunoreactivity in the frog sympathetic ganglia: non-identity with LRF decapeptide. Neuropeptides, in press, 1980.

Russell, J.T., Brownstein, M.J., and Gainer, H.: Liberation by trypsin of an arginine vasopressin-like peptide and neurophysin from a M_r 20,000 putative common precursor. Proc. Natl. Acad. Sci. USA 76: 6086-6090, 1979.

Russell, J.T., Brownstein, M.J., and Gainer, H.: Biosynthesis of vasopressin, oxytocin and neurophysins: Isolation and characterization of two common precursors (propressophysin and prooxyphysin). Endocrinology, in press, 1980.

Russell, J., Brownstein, M.J., and Gainer, H.: Time course of appearance and release of ^{35}S-cysteine-labeled neurophysins and peptides in the neurohypophysis. Brain Res., in press, 1980.

Russell, J., Brownstein, M.J., and Gainer, H.: ^{35}S-cysteine-labeled peptides transported to the neurohypophysis of adrenalectomized, lactating, and Brattleboro rats, Brain Res., in press, 1980.

Schaeffer, J.M., Schmechel, D.E., Conn, P.M., and Brownstein, M.J.: A simple and rapid method to isolate rat retinal cells for biochemical analysis. Neuropeptides, in press, 1980.

Young, W.S., Kuhar, M.J., Roth, J., and Brownstein, M.J.: Radiohistochemical localization of insulin receptors in the adult and developing rat brain. Neuropeptides, in press, 1980.

Loh, Y.P., Eskay, R.L., and Brownstein, M.J.: α-MSH-like peptides in rat brain: identification and changes in level during development. Biochem. Biophys. Res. Commun. 94: 916-923, 1980.

Hays, S.E., Beinfeld, M.C., Jensen, R.T., Goodwin, F.K., and Paul, S.M.: Demonstration of a putative receptor site for cholecystokinin in rat brain. Neuropeptides, in press, 1980.

Eskay, R.L., Furness, J., and Long, R.: Demonstration of immunoreactive substance P and somatostatin in human neural retina. Lancet, in press, 1980.

Oliver, C., Eskay, R.L., and Porter, J.C.: Developmental changes in brain TRH and in plasma and pituitary TSH and prolactin levels in the rat. Biol. Neonate 37: 145-152, 1980.

Skolnick, P., Paul, S., Zatz, M., and Eskay, R.: "Brain specific" benzodiazepine receptors are localized in the inner plexiform layer of rat retina. Eur. J. Pharmacol., in press, 1980.

Munemura, M., Eskay, R., and Kebabian, J.: Release of α-melanocyte-stimulating hormone from dispersed cells of the intermediate lobe of the rat pituitary gland: Involvement of catecholamines and adenosine 3',5'-monophosphate. Endocrinology 106: 1795-1803, 1980.

Munemura, M., Cote, T., Tsuruta, K., Eskay, R., and Kebabian, J.: The dopamine receptor in the intermediate lobe of the rat pituitary gland: Pharmacological characterization. Endocrinology, in press, 1980.

Cote, T., Munemura, M., Eskay, R. and Kebabian, J.: Biochemical identification of the β-adrenoceptor and evidence for the involvement of a cyclic AMP system in the β-adrenergic-induced release of α-melanocyte stimulating hormone in the intermediate lobe of the rat pituitary gland. Endocrinology, in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00242-06 LDP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Development of Sensitivity to the Feeling States of Others

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .28	PROFESSIONAL: .20	OTHER: .8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In a series of interrelated investigations (02132, 02133, 00273, 00275, and 02145) children's emotional development and the influences of various affective dimensions of rearing conditions on this development have been the subject of study. This project is the initial study of this program of research. Its focus is on children's responses to emotional distress in another person. Children are studied longitudinally in the second and third years of life. Mothers were trained to observe and report distress incidents in the natural environment of the child. Simulated distress incidents (by mother and by investigator) were also used. An initial sample of 24 families and a replication sample of 20 families have been studied longitudinally for nine months.

Developmental stages in responding to distress are identified between 10 and 24 months. Individual patterning in ways of responding to others' distress is evident by the end of the second year.

Project Description:

Emotional dimensions of the social environments in which children live have implications for children's functioning. The emotions and feelings of other persons provide many kinds of emotional stimuli, both positive and negative, which may be experienced by the child as isolated events, or as chronic conditions. In a series of interrelated investigations (02133, 00273, 00275, and 02145) children's emotional development and the influences of various affective dimensions of rearing conditions on this development have been the subject of study. This project is the initial study of this program of research. Its focus is on children's responses to emotional distress in another person. The research approach is longitudinal; each child is followed for nine months during the second and third years of life. Particular emphasis is on the emergence of, and developmental transitions in children's reactions to distress signals in others. Many data points are obtained in order to be able to observe, minutely, successive developmental changes.

Mothers were trained to observe and report on distress incidents in the natural environment of the child. Simulated distress incidents (by mother and by investigator) were also used. The details of the method have been described in the reports of previous years.

The first sample consists of 24 families with three age cohorts, one beginning at 10 months, one at 15 months, and one at 20 months. The second (replication) sample consists of 20 families beginning at 10 months.

The findings show stagelike developmental changes in responding to distress in others. Somewhere between 10 and 18 months of age, the majority of the children progress through developmental sequences that move from little apparent response or a global distress response to distress, to some form of positive reaching out toward and caring for another person in need. By the age of 1-1/2 to 2 years many children are able to perform well, a caregiver function. There are also clear individual differences in the form and manner of expression of these behaviors, the cognitive and affective accompaniment of prosocial behavior, and the frequency of concerned responding. Some children reach out to the mother with concern while for others the pattern of consoling is now routinized. Some perform their comforting acts with competence and confidence, while for others there is considerable hesitation and uncertainty. Moreover, the content of the prosocial response varies considerably. Not only are these children able to comfort another person by patting, hugging, or presenting an object but they have more sophisticated and elaborate means of trying to help other persons. They begin to express sympathetic concern verbally, they sometimes give suggestions about how to deal with a problem, they appear to try to cheer others up, and they are capable of trying alternative response forms when a given technique does not work. Many different non-empathetic patterns occur as well: children can also be variously oblivious, indifferent or avoidant of other's distress. Analyses are directed toward the kinds of learning processes that underlie the changes and individual differences in responding. A manuscript is in press. Further reports are in preparation.

This is a final report.

Publications:

Radke-Yarrow, M. R., Zahn-Waxler, C., & Chapman, M.: Prosocial Dispositions and Behavior. In Mussen, P. (Ed.): Manual of Child Psychology Volume on Personality and Social Development. New York, John Wiley & Sons, Inc. In press for 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00244-05 LDP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

A Behavior Profile for Child Psychopathology and Social Competence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.: Thomas M. Achenbach Research Psychologist LDP NIMH

COOPERATING UNITS (if any)
25 Child guidance clinics and other child mental health agencies

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.15	.60

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the research is to develop a standardized behavioral profile for child psychopathology and social competence to be used for research on etiology, treatment effects, epidemiology, and prognosis, as well as for clinical purposes. Parents of disturbed and normal children fill out a behavior checklist describing their child's behavior problems and strengths. Data from the checklists are factor analyzed to identify items that group together within various age and sex groups. The factors are employed as scales of the behavior profile. Empirically obtained distributions of scales on each score provide norms with which the scores of individual children can be compared, and behavior profiles obtained on the same child at different times can be used to assess changes in behavior.

PHS-6040
(Rev. 10-76)

Project Description:

An acute problem in research on the etiology, epidemiology, treatment, and prognosis of child psychopathology is the lack of a standardized and objective system for assessing childhood behavioral disorders. The purpose of the present research is to develop the Child Behavior Profile, an instrument that employs readily obtainable data to portray children's behavioral problems and social competencies in a standardized and objective format.

The data are obtained with the Child Behavior Checklist, which is filled out by parents of children aged 4 to 16 years. Behavior problems (e.g., hyperactivity, disobedience, fears, bedwetting) are scored on three-point scales according to intensity and frequency. Social competence items include reports of participation in sports, hobbies, and organizations, special skills, number of friends and amount of interaction with them, and school performance. The Behavior Checklist is the product of several pilot editions that have been tried out in child guidance clinics and have been progressively revised on the basis of feedback from clinicians, patients, and nonprofessionals.

The data for the Child Behavior Profile have been obtained from Behavior Checklists filled out by parents of children seen in some 25 mental health settings, plus randomly selected parents of normal children. Factor analysis is employed to identify items that tend to occur together in children grouped by age and sex. Several hundred subjects are included in each sample (e.g., boys aged 6 to 11, girls aged 6 to 11, boys aged 12 to 16, etc.)

The groupings of items obtained through factor analysis are used to define the scales of the standardized profile for each age and sex group, and the empirically obtained distributions of scores on each scale provide norms with which the scores of individual children can be compared. The Profile can be used to classify children for purposes of research on etiology, treatment effects, epidemiology, and prognosis, as well as to provide clinicians with a standardized tool for describing and communicating about psychopathology.

Profiles have been completed for boys at ages 4 to 5, 6 to 11, and 12 to 16, and for girls at ages 6 to 11 and 12 to 16, with Profiles for girls aged 4-5 expected to be completed soon. Average one-week test-retest reliability coefficients have been .89 for 6- to 11-year-old boys, .82 for 12- to 16-year-old boys, .88 for 6- to 11-year-old girls, and .90 for 12- to 16-year-old girls. Average interparent agreement coefficients have been .74 for 6- to 11-year-old boys, .79 for 12- to 16-year-old boys, .63 for 6- to 11-year-old girls, and .54 for 12- to 16-year-old girls. Significant stability has also been found in Profiles scored before and after outpatient psychiatric treatment, over a follow-up interval of 18 months. The stability coefficients averaged .63 for 6- to 11-year-old boys, .54 for 12- to 16-year-old boys, .55 for 6- to 11-year-old girls, and .42 for the 12- to 16-year-old girls.

Despite decreases in the number of behavior problems reported after treatment, the patterning of behavior remained relatively stable, as reflected in significant Q correlations between the shapes of Profiles obtained at intake and follow-up. The edition of the Profile for 4- and 5-year-old girls will be completed as soon as enough cases of this seldom referred group are accumulated.

This is a final report.

Publications

Achenbach, T. M. The DSM-III in light of empirical research on the classification of child psychopthology. J. Am. Acad. Child Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00245-04 LDP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Objective Identification of the Hyperkinetic Syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH
Other	Gerald L. Brown	Research Psychiatrist	BPB	NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, MD 20205

TOTAL MANYEARS: .10	PROFESSIONAL: .05	OTHER: .05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study is to identify objectively the behavioral patterns associated with findings obtained through intensive medical workups of children referred for hyperactivity. Both parents of a referred child independently complete a behavior checklist from which a standardized behavior profile is derived. Degree of inter-parent agreement is computed and the profiles are analyzed in order to classify children according to types of behavior patterns. Classification of children in this way is then compared with ratings of ward behavior, with the outcomes of the medical work-up, and with the effects of stimulant drugs to determine whether the behavioral typology corresponds to characteristics of the children identified clinically.

PHS-6040
(Rev. 10-76)

Project Description

The "hyperkinetic syndrome" is today the most widely publicized of childhood disorders. There has been a tremendous increase in the number of children labelled as hyperkinetic and a great deal of controversy over such diverse approaches to treatment as chemotherapy, dietary manipulations, behavior therapy, and laissez faire permissiveness. Etiological hypotheses show a parallel diversity, with minimal cerebral dysfunction, allergies, anxiety, inappropriate reinforcers, constitutional characteristics of temperament, and creative nonconformity all being implicated by one authority or another.

One reason for the diversity of treatment and etiological hypotheses is that there is no objective way to define the behavioral phenotype of hyperkinesis. Since most children are from time to time too active for the tastes of their parents and teachers, and since the terms "hyperactivity" and "hyperkinesis" have gained such wide currency, reports of high activity level alone cannot be regarded as pathognomic. Identifying children who share some common etiological factor with respect to activity level or who would benefit from a particular treatment has thus been made difficult by the excessively broad application of the concept of hyperkinesis.

In order to determine whether there is an identifiable behavioral phenotype corresponding to a clinical syndrome which may include organic anomalies and which may benefit from chemotherapy, collaborative research is being carried out with Dr. G. L. Brown of the NIMH Biological Psychiatry Branch in connection with a study of children referred for hyperactivity (cf. Biological Psychiatry Branch Project Number Z01 MH 0100-04BP). Both parents of each referred child independently complete the Child Behavior Checklist (described in report on Project Number Z01 MH 00144-03 LDP). An extensive life history is also obtained and the children are admitted to the NIH Clinical Center for several weeks of medical, behavioral, and pharmacological assessment. Without knowledge of the results of these assessments, we are scoring the behavior profile patterns from the Checklist completed by each parent. Interparent reliability is assessed and the separate profiles as well as a composite profile based on the checklists completed by both parents are analyzed to identify particular types of profile patterns. These are being compared with the results of the clinical work-ups to determine whether the types of profile patterns correlate with differential clinical findings and with ratings of ward behavior. Preliminary findings indicate a clearcut behavioral phenotype of hyperactivity and high correlations between parent ratings on our Checklist and one developed by C. Keith Conners for use in evaluating the effectiveness of drug treatments with children.

If further relationships between the behavior profile patterns and clinical findings are confirmed, the profile patterns can be employed to form homogeneous groups for research purposes. As specific etiologies and/or treatments are discovered for these groups, the profile patterns can be employed by clinicians to guide diagnosis and the choice of treatment.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00246-05 LDP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
A Follow-up Study of Children Differing in Behavior Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)
3 Child Guidance Clinics in New Haven and Waterbury, Connecticut

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION
NIMH ADAMHA NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

TOTAL MANYEARS: .10	PROFESSIONAL: .05	OTHER: .05

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the research are (1) to determine the stability of behavior profiles based on behavior checklists describing disturbed children, and (2) to determine the prognosis for children differing in initial profile patterns. Parents of children, for whom Child Behavior Checklists were filled out at intake, filled out Checklists describing their children's behavior problems and social competencies following treatment. Behavior profiles based on the follow-up Checklists are being compared with those based on the initial Checklists to determine the stability and differential prognosis for different types of profiles in various age, sex, and socioeconomic groups.

PHS-6040
(Rev. 10-76)

Project Description:

Effective utilization of mental health services to children is greatly handicaped by the current lack of knowledge about prognosis for various behavior problems. Although the demand for services to children far exceeds the supply, there is no objective basis for selectively assigning treatment to those who will benefit most, because it is not known which children will actually be helped by treatment, which ones will improve without treatment, and which ones will fail to be helped by current treatment approaches.

A primary obstacle to research on prognosis has been the lack of a standardized and reliable diagnostic-classification system for childhood behavior disorders. As described in the report on Project Number Z01 MH 00244-05 LDP, the investigators have developed a standardized behavior profile for child psychopathology and social competence. It is expected that children having different profile patterns will differ in their prognosis under various conditions of treatment and nontreatment.

To test both the stability and the predictive power of the profile patterns, a follow-up was conducted of 200 children for whom Child Behavior Checklists were filled out in three clinics at intake. Parents of these children were asked to fill out Checklists describing their children's behavior problems and social competencies following termination with the clinics. Statistical analyses have shown that children who differed in their initial profiles subsequently differ with respect both to their patterns of problems and competencies and to their general adjustment. The findings also indicate statistically significant stability in the patterns of reported problems despite significant declines in the number of problems reported.

Further analyses are expected to show whether initial profile patterns are differentially related to treatment outcome and whether the patterns are useful for determining prognosis under typical clinic procedures. The data will also be compared with outcome data for children seen in other settings and those receiving systematic assignment to particular treatments.

The data obtained in Project Z01 MH 00269-03 are being included in this project.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00247-05 LDP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Relations between Cognitive and Behavioral Patterns in Children with Endocrine Abnormalities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

National Naval Medical Center
Bethesda, Maryland 20014

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Behavior checklists and measures of cognitive functioning are being obtained on normal controls and children who have thyroid and sex hormone abnormalities in order to determine whether hormone levels affect patterns of cognitive and behavioral adaptation and the relationships between them. The study is designed to test the hypothesis that increases in sex hormone level affect patterns of cognitive functioning by improving automatization abilities at the expense of ability to engage in complex perceptual restructuring. It is also designed to determine whether premature craniosynostonis noted in hyperthyroid children interferes with cognitive functioning.

PHS-6040
(Rev. 10-76)

Project Description

One of the most neglected aspects of research on childhood behavior disorders is the interface between cognitive functioning and maladaptive behavior. Research and theory tend to focus on cognitive disabilities as one substantive area and "emotional" problems as a separate substantive area. However, from a developmental perspective, cognitive functioning is intimately interwoven with the social, biological, and emotional aspects of adaptive processes. On the one hand, a child's level of cognitive development is likely to set limits on the types of adaptive strategies available to him or her. On the other hand, social, biological, emotional, and motivational factors are likely to influence the degree to which children make use of their cognitive capacity.

Work by other investigators has suggested that sex hormone levels affect the action of neurotransmitters in the brain, and that these, in turn, affect the patterning of cognitive abilities. In particular, it has been found that high levels of sex hormones, especially estrogens, are positively correlated with performance on automatization tasks requiring quick, accurate responses, but are negatively correlated with performance on tasks that require complex perceptual restructuring. Our study is designed to test this hypothesis by comparing cognitive functioning and behavioral patterning before and after treatment in children who receive sex hormone supplementation for various endocrine abnormalities. Children with thyroid abnormalities are also being studied because clinical observations have revealed premature craniosynostonis in these children, and it is important to determine whether cognitive and behavioral patterns are affected by this condition. The children and their sibling controls are recruited by Dr. Roger Johnsonbaugh through his pediatric endocrinology service at the Naval Medical Center.

Our measures of cognitive functioning include the Wechsler Intelligence Scale for Children (WISC), the Embedded Figures Test, a measure of speed of color naming, and a measure of speed of object naming. Differences between perceptual restructuring measures (WISC Block Design, Embedded Figures Test) and automatization measures (color naming, object naming) will be compared at two testings to determine whether changes in hormone levels after medication cause changes in cognitive patterning that do not occur in normal, unmedicated control subjects.

Preliminary findings indicate some differences in cognitive patterning between children with thyroid abnormalities and their normal controls, but not enough subjects have yet been tested to determine the generality of these differences or to draw conclusions about the effects of sex hormones. The study will be continued if additional subject samples can be obtained.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00250-05 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Information Processing in Premature and Full-term Infants at the Perinatal Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sarah L. Friedman	Guest Worker	LDP NIMH
OTHER:	Blanche S. Jacobs	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Washington Hospital Center, Washington, D. C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.20	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study sensory function and temperament were compared in infants varying in gestational and medical status at birth. The infants who were studied included low medical risk preterms at their expected date of delivery, healthy full-term neonates, and post-term neonates. Sensory function was evaluated by measures of response to novelty, level of responsiveness, and response decrement collected when the infants were responding to tactile, auditory, and visual stimulation. Temperament was assessed on the basis of the spontaneous behaviors of the babies, their responsiveness to soothing when they cried, and their responsiveness during a neurological examination. Temperament was described in terms of state organization, irritability, soothability, and activity level. Preterms were different from full-terms on auditory, visual and temperamental measures. Post-term infants differed from full-terms on some temperamental measures.

PHS-6040
(Rev. 10-76)

Project Description

Most pregnancies last between 38 and 42 weeks. Some, however, terminate much earlier or later. Medical technology has made it possible for infants born as early as at 28 weeks or as late as at 50 weeks of gestation to survive. This is certainly an impressive medical accomplishment; yet, the psychological outcomes of two short or too long periods of gestation are not fully understood. It is not obvious that the central nervous system of a preterm organism is ready to benefit from extrauterine stimulation. Nor is it obvious that a post-term organism has not suffered because of its not being exposed to an extrauterine environment at a possibly critical period for some aspects of development. Birth not at term is often but not always associated with medical complications. This raises the question about the role of illness in early development. It is possible that a newborn who spends much energy in a struggle for biological survival has very little energy left for psychological development.

Evidence from late infancy and childhood indicates that children who were not born at term are at a greater than average risk for intellective and behavioral deficits. In this study we explore some precursors of intellective and behavioral deficits in early infancy -- in the days after birth or at the expected date of birth (in the case of preterms). Knowledge of specific weaknesses and strengths at this early stage should make it possible to rationally plan early intervention programs to stimulate those aspects of development which show deficits due to birth not at term.

The psychological functions which were studied are of two classes: Sensory processing as an index of the degree of cortical maturity and integrity, and temperament. Sensory processing was assessed in the tactile, auditory and visual modalities. In the examination for tactile processing, the infant's left cheek was stroked for 2 secs. repeatedly with a nylon filament. There was a random interstimulus interval. When the infant reached a pre-determined response decrement criterion, direction of stroking was changed. Stroking continued until the same criterion was reached. Behavioral data were collected that provided measures of response to novelty, level of responsiveness and response decrement. In the auditory information processing examination, the infant was presented with one of two tones (500 cps or 1500 cps) at 80 db. The procedures and measures were equivalent to those described for the tactile modality. In the visual processing examination, first one then the other of two stimuli (revolving, lighted, colored plexiglass boxes) were presented repeatedly for 30-second periods starting with the infant's first look at the stimulus. Visual attention data were collected.

Temperaments of children are believed to influence parent-child relationships. These relationships in turn are believed to influence the social and cognitive development of children. We were interested in finding out whether non-term infants were temperamentally different from full-term infants. Such differences could feed into abnormal infant-caregiver interaction and might lead to child abuse.

Temperament was assessed by the state of arousal and behaviors of the infant when he/she was free of discomfort, when he/she was crying and responding to soothing, and during a neurological examination. During 18 minutes the infant was observed for periods of 5 seconds, followed by 10-second periods in which the observer coded the infant's behaviors. Infant behaviors which were coded included state of arousal, body, limb, and facial movements as well as looking activity and vocalizations. If the infant cried, an experimenter intervened in a progressive manner in an attempt to soothe the infant. A brief neurological exam tested for the neck muscle control, arm and leg recoil, arm and leg traction, head raising, arm release, the Galant reflex, the sucking ability, the rooting response, foot withdrawal and the moro reflex. At five specified times during the exam, the arousal state of the infant was coded. Twenty-three full-term, 45 preterm, and 9 post-term infants were studied.

Full-term and pre-term infants were equally responsive to the tactile stimulation. Full-term infants were somewhat ($p < .10$) quicker to respond to the initiation of auditory stimulation and responded more vigorously on the first few trials ($p < .05$). Full-terms were also quicker to respond to the initial presentation of the visual stimulus ($p < .05$) and took less time to reach the visual response decrement criterion ($p < .05$). Post-term subjects were not deficient relative to the full-term subjects in their tactile, auditory or visual responsiveness.

Temperament data show that the full term subjects were of "easier" temperamental dispositions than preterms. They had less labile state organization ($p < .001$), were fussing and crying less ($p < .02$, $p < .04$), and were less active ($p < .02$) throughout the observations of spontaneous behaviors. They were also less irritable and easier to soothe during the neurological exam ($p < .01$, $p < .01$). Full-terms were also temperamentally easier than post terms. They had a less labile state organization ($p < .02$), and they were less irritated by tactile stimulation ($p < .01$) and by the neurological exam ($p < .04$). However, once upset, they were more difficult to soothe than the post-term subjects ($p < .04$). Our results show that preterms, and to a lesser extent post-terms, present themselves as temperamentally more difficult than full-term infants.

This is a final report.

Publications:

Friedman, S. L., Jacobs, B. S., and Werthmann, M. W., Jr. Tactile, auditory, and visual processing in pre-term, full-term, and post-term infants at the neonatal period. In Friedman, S. L. and Sigman, M. (Eds.): Pre-term and Post-term Birth: Relevance to Optimal Psychological Development. San Francisco, Academic Press, 1980, In Press.

Barnet, A. B., Friedman, S. L., Weiss, I. P., Ohlrich, E. S., Shanks, B., and Lodge, A. A longitudinal study of infants' EEG visual evoked potentials. Electroencephalogr. Clin. Neurophysiol., In Press.

Friedman, S. L., Jacobs, B. A., and Werthmann, M. W., Jr. Preterms of low medical risk: Spontaneous behaviors and soothability at expected date of birth. Infant Behav. Dev., In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00253-05 LDP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Behavioral Studies of Seriously Ill Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth J. Susman	Senior Staff Fellow	LDP NIMH
OTHER:	Barbara E. Strope	Social Science Analyst	LDP NIMH
	Stephen P. Hersh	Director, Child and Adolescence Service	St. Elizabeth's Hospital NIMH
	Arthur S. Levine	Chief	POB NCI
	Philip A. Pizzo	Senior Investigator	POB NCI

COOPERATING UNITS (if any)

Pediatric Oncology Branch, National Cancer Institute

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.25	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Physical well-being is an assumed, though generally unmeasured, condition in studies of the child and adolescent's psychological development and behavior. There is little developmental inquiry concerning the effects of absence or loss of physical well-being on child development, despite the reality that acute or chronic ill health from disease or accident is, to a great or small degree, part of the life of every child. This project is concerned with the psychological and social impact of serious illness (cancer) on children and those in their social network (namely their families and caregivers). Areas of research include coping abilities, the influence of the social system of the child, and the mutual influences of the stressed children and those in their social network. Observations, interviews, and parent and nurse ratings were used to investigate these phenomena.

PHS-6040
(Rev. 10-76)

Project Description

The purpose of the project was to assess the impact of a life-threatening illness (cancer) on children, adolescents, and their families. The research participants were patients and their families on the Pediatric Oncology Branch of the National Cancer Institute. The sample consisted of children 18 months to 21 years, with metastatic solid tumors and lymphomas that had failed to respond to conventional therapeutic regimens. These children were research participants in a NCI study designed to test the utility of a protected environment (Laminar Air Flow room) for reducing infections while receiving experimental, intensive chemotherapy. A protected environment (P.E.) limits the amount of social, emotional, and perceptual stimulation available to the patient.

The sample consisted of two groups of children. For the older group of children and adolescents ($\underline{n}$ = 19), data collection methods included systematic observation, interviews, and parent and nurse ratings. The younger group of children ($\underline{n}$ = 4) were observed twice a day, four times a week.

Findings from the observational data indicate that, while the child is confined to the P.E., changes in behavior are related to, but not solely determined by changes in physiological status. Once chemotherapy affects individual physiological functioning, there is increased patient withdrawal and decreased activity level. However, as physiological integrity is restored, social behaviors do not improve. Along with this general trend is the high variability among patients in kind and direction of change. Significant factors influencing patient behavior changes are the level and kind of caregiver and family interaction with the child.

Reports of these findings have been submitted for publication. This is a final report.

Publications:

Susman, E. J., Hollenbeck, A. R., Nannis, E. D., Strope, B. E., Hersh, S. P., Levine, A. S., and Pizzo, P. A. Interactions between primary caregivers and children with cancer: A methodology for systematic observation in a hospital setting. In Bugen, L. (Ed.): Death and Dying: Theory, Research, and Practice. Dubuque, Iowa: W. C. Brown & Co., 1979, pp 213-224.

Levine, A. S., Artiss, K., and Susman, E. J. The Impact of Caring for Children with Cancer on Doctors and Nurses. Proceedings of the American Cancer Society Conference on the Care of the Child with Cancer, 1979, pp. 137-143.

Susman, E. J., Hollenbeck, A. H., Strope, B. E., Hersh, S. P., Levine, A. S., Pizzo, P. A. Separation-deprivation and Childhood Cancer: A Conceptual Reevaluation. In Kellerman, J. (Ed.), Psychological Aspects of Childhood Cancer. Springfield, Illinois, Charles C. Thomas, in press.

Susman, E. J., Hollenbeck, A. H., Nannis, E. D., and Strope, B. E. A Developmental Perspective on Psychosocial Aspects of Childhood Cancer. In Schulman, J. L. and Kupst, M. J. (Eds.): The Child with Cancer - Clinical Approaches to Psychosocial Care. Springfield, Illinois, Charles C. Thomas, 1980, pp. 129-142.

Susman, E. J., Pizzo, P. A., and Poplack, D. Coping With Adolescent Cancer: Living With the Aftermath. In Ahmed, P. (Ed.): Coping With Cancer. New York: Elsevier-North Holland Publishers, in press.

Hollenbeck, A. R., Susman, E. J., Nannis, E. D., Strope, B. E., Hersh, S. P., Levine, A. S., and Pizzo, P. A. Children with serious illness: Behavioral correlates of separation and isolation. Child Psychol. Hum. Dev., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00255-04 LDP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Maternal Imprisonment: Psychological Effects on Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Velma LaPoint	Staff Fellow	LDP NIMH
OTHER: Marian R. Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)
Maryland Division of Corrections, Baltimore, MD
Maryland Division of Parole and Probation, Baltimore, MD
National Institute of Justice, (LEAA),Department of Justice, Washington, DC

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA NIH, Bethesda, MD 20205

TOTAL ~~MANYEARS~~: staff years 1.40	PROFESSIONAL: 1.10	OTHER: .30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The lack and/or loss of stable and supportive parenting when a mother is imprisoned represents a psychological at risk situation for children. The purpose of the study is to identify psychological consequences for children whose mothers are imprisoned. Risk for children is compounded when the reasons for strained, inadequate parenting are themselves indicative of psychological problems and stress of mothers. Children's attitudes toward self, affective state, interpersonal relations with peers, adult authority figures, and relations with their mothers are studied. Research participants from Baltimore are 41 imprisoned mothers, children, children's caregivers. Mothers and children from single-parented families in the same community comprise the comparison groups: mothers who are currently on probation, and mothers with no previous involvement in the criminal justice system. The groups are comparable racially (Black) and socioeconomically. Data are obtained in home contacts, mother-discussion groups, mother-child dyads and group interactions. Secondary data are obtained from school records.

Project Description

The loss of stable and supportive parenting places children at risk for mental health problems. Risk for the child is compounded when the reasons for inadequate parenting are themselves indicative of psychological problems of the parents. Children whose mothers are imprisoned constitute such a group at risk. The purpose of this study is to identify psychological correlates and consequences for children of maternal imprisonment. Relationships of mother, child, and caregiver, the child's experiences in the process of mother's incarceration, and the child's problems and his/her abilities to cope with daily demands of living are investigated. An examination of the child's supportive network is included.

Research participants from Baltimore, Maryland are 41 imprisoned mothers at the Maryland Correctional Institution for Women, Jessup, Maryland and their children who reside with relatives in their homes in Baltimore. Children are between 5 and 12 years old. Mothers and their children from single parented families comprise two comparison groups: mothers who are currently on probation in the criminal justice system and mothers who have no previous involvement in the criminal justice system.

Data are obtained in (a) home contacts, (b) mother discussion groups, (c) mother-child dyads, and (d) group interactions. A secondary data source is school records of children's academic and behavioral competence. The prison setting is utilized when data collection relates to incarcerated mothers in discussion groups or with their children. A community (church) setting is used for other data collection.

Maternal data are obtained on mother's roles and attitudes toward their children and attitudes toward themselves as mothers. Assessments are made of mother-child relationships, child affective states, attitudes toward self and adult authority figures, and problems and competencies in coping with day-to-day demands.

Findings:

A variety of maternal behaviors and mother-child relations existed prior to and during imprisonment: Children were often recipients of multiple mothering from the mothers and maternal grandmothers. Half of the mothers had had caregiving responsibility for children prior to incarceration, half had little involvement with their children. Reasons for minimal involvement included: factors and handicaps associated with adolescent pregnancy and parenting, involvement in activities which competed with child care responsibilities, and previous incarcerations. For these children, incarceration did not bring drastic changes in the child's responsible parenting figure. Usually a continuing caregiver remained during maternal incarceration.

In current relationships between mothers and children, three themes emerged which were descriptive of maternal roles during incarceration: (1) mothers' emphasis on "instrumental" material aspects of parenting which, in some cases, was mixed with superficialities; (2) mothers' remoteness from and lack of knowledge of the details of their children's lives; and (3) mothers' helplessness and inadequacy when confronted with issues of discipline and respect from the child, and feelings of guilt concerning their offender-status and its impact on their children.

Observations of mother-child interactions were analyzed in the areas of intimacy, nature of authority roles and affective qualities. Half of the pairs showed signs of being quite intimate in their ability to share feelings and serious concerns. Others lacked intimacy and were quite superficial. Half of the pairs displayed varying degrees of role confusion in which children assumed the parental role and mothers assumed a dependent role. Other pairs were more like siblings and peers to each other. A fourth of the children expressed clear negative feelings in the relationship, often very skillfully punishing their mothers.

The observed ability of mother and child to communicate intimately was significantly related to ratings of responsible maternal roles prior to prison and to mother's competence within the prison. Observed positive interactions between mother and child were related to ratings of mother's comprehension on the impact of her status on the child. Incompetence in prison adjustment was related to role reversal. Intimate communication, positive affect, and non-reversal of roles were positively related.

Analysis of children's behavioral competencies at home and school are underway. Problems for children include anxious and withdrawn behaviors, disciplinary and acting out problems, and poor academic achievement. Data collection on comparison families is underway.

Findings from the study will provide information about children's and families' coping with stress. Findings also have implications regarding policies for incarcerated mothers and their children. A wide range of social service and correctional policies are being considered yet there is little known about the nature of mother-child relationships of incarcerated mothers and their children or about the effects of maternal imprisonment on children.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00256-05 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Expressions of Affect: Children's Age and Sex-role Expectations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karabelle A. L. Pizzigati	Guest Worker	LDP NIMH
OTHER:	Gale Inoff	Psychologist	LDP NIMH

COOPERATING UNITS (if any)
Green Acres School, Rockville, Maryland

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .10	PROFESSIONAL: .05	OTHER: .05

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children's judgments with regard to sex-role-related personality traits were investigated. Children, ages 5-13 years, viewed videotaped skits portraying traits stereotypically associated with one sex or the other (e.g., gentleness, stoicism). The children evaluated themselves, considered peers in terms of the traits portrayed, and assessed the social desirability of the traits. The findings indicated only modest concordance between the children's perceptions of themselves (as well as their peers) and the stereotypes. No age patterns were found consistently across traits. The self ratings were related to ratings of social desirability of the traits, especially at the youngest age period. It seems that, when cued in by the task or instructions, children can accurately report the content of stereotypes and use them "appropriately". When not cued in to stereotyping, however, they may or may not use stereotypes to organize their information processing. Investigators need further to identify the characteristics of situations which elicit the use of stereotypes.

PHS-6040
(Rev. 10-76)

Project Description

The purpose of this project was to investigate the importance of the gender dimension in children's perceptions of themselves and their peers. The sample consisted of 150 middle and upper-middle class boys and girls, ages 5-13 years, attending a private school. Each participant individually viewed 10 videotapes portraying the following traits: gentleness, fickleness, emotional expressiveness, dependency, nurturance, stoicism, messiness, assertiveness, independence, and boastfulness. Evidence from the literature and from pilot data suggests that these traits relate to sex role attitudes. For each trait, the children were asked to describe the portrayal, rate themselves on the trait portrayed, nominate two peers who exhibit the trait, and evaluate the social desirability of the trait.

When looking for evidence of sex role stereotypes, one would expect a clustering of traits that represent each sex role. In the children's self ratings, although the correlations which replicated across age groups were almost always masculine traits correlating with masculine traits or feminine traits correlating with feminine traits, the traits did not fall into sex role clusters. There was no evidence that masculinity and femininity are anchors of a bipolar dimension. A recent view is that masculinity and femininity are independent dimensions and that androgyny is a possible (as well as an adaptive) orientation; we, however, did not find evidence of these dimensions

From traditional stereotypes one might predict that, with age, boys would become more masculine and less feminine and that girls would become more feminine and less masculine. Cross-sectional analyses for age differences between groups lent meager support for this hypothesis. There was no consistent pattern across traits. Only when the requirements for a sex role were made less stringent did we find significant age differences--boys decreasing in self-ascribed femininity with age. No masculine sex role was found even when the requirements were eased.

Although clustering of traits and age differences are not necessary to infer that the self ratings are in accordance with a stereotype, one would expect differences between the sexes. There were more sex differences in self ratings than one would expect by chance, but the results were not compelling. Almost all of these sex differences were on the feminine traits. This finding is compatible with results of other studies concerning avoidance of sex-inappropriate activities and differential socialization of such behavior. Since girls more readily engage in cross-sex behavior, it might be expected that sex differences would be attenuated for the masculine traits.

Self ratings also were analyzed in relation to ratings of social desirability of the traits. The self ratings were correlated with social desirability, especially at the youngest age period.

When the children named peers who they felt exhibited each trait, there was some concordance with the stereotype, but the most likely response was to name same-sex peers, regardless of the trait.

In children's self perceptions and peer nominations, sex role stereotyping was not readily apparent. However, when children were asked whether each trait was "for boys" or "for girls", there was considerable sex typing in line with the larger society's stereotype. It appears that, when told to do so, children can accurately report the content of stereotypes and use them "appropriately". When not cued in by task or instructional sets, they may or may not use stereotypes to organize their information processing. The task presented to the children seems to be an instance in which most of the children did not spontaneously use sex role stereotypes. Investigators need further to identify the characteristics of situations which elicit the use of stereotypes.

A manuscript of this study has been submitted for publication.

This is a final report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00257-04 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	David G. Poplack	Senior Investigator	POB NCI
	Van S. Hubbard	Clinical Associate	NIAMD

COOPERATING UNITS (if any)
NCI
NIAMDD
Los Angeles Children's Hospital

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.60	.50	.10

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of CNS treatment (cranial irradiation and intrathecal chemotherapy) on the cognitive and perceptual functioning of children with leukemia are investigated. One study compared a group of leukemic children in remission with a matched group of sibs on measures from a battery of psychological tests (Wechsler, Bender-Gestalt and Graham-Kendall). These groups are followed-up 12-15 months after the initial assessment. A sample of leukemia patients who have not received CNS treatment and their sibs and a sample of cystic fibrosis patients and their sibs are also being assessed using these procedures to control for effects other than CNS treatment. A second study deals with another sample studied prospectively. In this instance children in remission from leukemia are evaluated on the same battery of psychological tests as in the first study. They are tested just prior to prophylactic CNS treatment and are re-evaluated one year later to determine if any possible brain changes brought on by the CNS treatment have resulted in learning disabilities and diminished intellectual functioning. The healthy siblings of the leukemics receiving CNS treatment, who are being studied retrospectively, will be used as a control group to check testing effects.

PHS-6040
(Rev. 10-76)

Project Description

The chance of survival from childhood leukemia has increased greatly over the past 10 years because of the utilization of new treatment procedures. The main change in treatment that has led to this improved outlook is the inclusion of prophylactic measures consisting of a series of cranial irradiations and intrathecal (spinal) injections of powerful chemical agents.

The purpose of the proposed research is to determine if CNS treatment has deleterious effects on intellectual functioning and to evaluate the nature and extent of these effects. Two studies are in progress. One study involves evaluation of intellectual functioning only after CNS treatment (post-treatment study); in the other study intellectual performance prior to CNS treatment is compared to performance subsequent to treatment.

Because of the age range of our subjects, the three Wechsler Intelligence Tests are used: WPPSI (ages 4-6), WISC-R (ages 6-16), WAIS (17 years of age and over).

Study I. Post-treatment study

The sample consists of 34 children who have been in remisssion for 2 to 5-1/2 years from acute lymphocytic leukemia (ALL), and a sibling control group. The mean age of the children at the time of assessment was 11 years with a range of 4 to 23. Thirteen leukemia patients who were treated for their leukemia without the use of CNS treatment and their siblings served as a control group. All children were administered a Wechsler Intelligence Test and the Bender-Gestalt.

For most comparisons involving the central nervous system treated group,the siblings performed at a significantly higher level of functioning than the patients. The mean Full Scale IQ of 99 for patients and 112 for siblings indicates that the patients are operating at an average and the siblings at a bright average level of intellectual functioning. There were no IQ differences between the patients and the siblings for the non-CNS treated patient group.

Other analyses of these data have shown that the longer the time interval between central nervous system treatment and the psychological assessment the greater the IQ decrement, and the younger the child at the time treatment was initiated the greater the apparent IQ loss. The variables "time interval between CNS treatment and assessment" and the "age of the child at the time treatment was started" were combined, using a ratio, into a new variable which reflected the "percentage of life lived since the initiation of treatment." This "percentage of life lived since treatment" variable was even more strongly related to IQ decrement than the previous two separate variables. There was also evidence that brighter children were significnatly more affected by the CNS treatment than the children of average potential.

Analyses are currently under way to try and develop diagnostic indices concerning brain damage from the Wechsler tests using the CNS treated patients as a criterion group.

Study II. Prospective Study

A sample of 60 children with acute lymphocytic leukemia, who receive prophylactic CNS treatment, are being administered the same psychological evaluation described in the Post-treatment study. This assessment is made before CNS treatment and one year after treatment. This interval of one year should be sufficient to allow for loss in learning abilities if such losses occur as the result of treatment. The sibling group of leukemic patients receiving CNS treatment who were a part of the Post-treatment study will be used as a control group for this study to partial out any practice effects that may contribute to the follow-up IQ scores among the patients.

Half of this sample will be between 3-1/2 and 6 years of age and half will be between 6 and 16 years of age. This age distribution will provide a good basis for evaluating whether age at the time of CNS treatment is a factor in contributing to the degree of intellectual deficit that may occur. Initial testing has been done in about 40 cases and the one year follow-up in about 20 cases.

Publications:

Moss, H. A., and Nannis, E. D. Psychological Effects of Central Nevous System (CNS) Treatment of Children with Acute Lymphocytic Leukemia (ALL). In Kellerman, J. (Ed.): Psychosocial Aspects of Childhood Cancer. Springfield, Charles C. Thomas, Publisher. In press.

Appendix to Project Number Z01 MH 00257-04

Contract #263-MD-001195 for $1480 has been used to reimburse testers, $40 per case. The tests given are the WISC. The work involved is the equivalent of 1/20 man years.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00259-04 LDP

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Relations between Parent, Teacher, and Clinician Reports of Child Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Woodburn Center for Community Mental Health, Annandale, Va.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL staff years: .20	PROFESSIONAL: .10	OTHER: .10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relationships between parent, teacher, and clinician reports of the behavioral problems and competencies of children referred for outpatient mental health services; (2) to determine the relationship between children's presenting behavioral patterns as reported by these sources and the outcome of their clinic contacts. At intake, and again following termination with the clinic, parents and a clinician filled out the Child Behavior Checklist, while the child's teacher filled out a Teacher Report form. Child Behavior Profiles scored from these data have been compared to identify agreements and disagreements between parents, teachers, and the clinician. The relationship of outcome to intake Profile patterns, demographic variables, and other characteristics of the children will also be assessed in order to identify predictors of poor outcome. If children who typically have poor outcomes can be identified, new treatment strategies may be developed to help them.

Project Description:

Little is known about the relationships between client characteristics and the effectiveness of existing treatments for child psychopathology. One of the purposes of the Child Behavior Profile (Project No. Z01 MH 00244-05 LDP) is to differentiate among children who differ in their responses to various treatment modalities. This study is designed to determine the degree of agreement and the nature of disagreements between Profiles scored from Child Behavior Checklists completed by children's parents, their teachers, and a clinician who did an intake and follow-up evaluation but was not involved in treatment. It is also designed to determine whether classification of children according to Profile patterns derived from reports by each type of informant separately and/or in composite is predictive of outcome in response to the child mental health services offered by a community mental health center.

In addition to the Behavior Profiles, the intake clinician obtained data on family composition, demographic characteristics, previous mental health contacts, degree of parent motivation for help, and the problems considered most serious by the parents. Six months after intake into the clinic, the intake clinician contacted the family to obtain Behavior Checklists describing the child's current status, plus data on changes in the family, the family's reactions to the services they have received, and possible needs for further services.

If children who have poor outcomes can be discriminated from those who have good outcomes on the basis of intake data, then new treatment strategies can be developed for helping children who are not likely to benefit from mental health services as currently offered. Initial analyses show correlations averaging about .80 between Child Behavior Profile scores scored from Checklists filled out by the clinician and the children's mothers at intake and about .75 at follow-up; correlations averaging about .41 between the clinician's ratings and teachers' ratings at intake and about .51 at follow-up; and correlations averaging about .28 between teachers' ratings and mothers' ratings at intake and negligible correlations at follow-up. Categorization of Profiles in terms of cluster-analytically based typologies has shown agreement ranging from 70 to 95% in profile patterns scored independently from Checklists filled out by the clinician and the children's mothers. Further analyses will be made to identify the best predictors of outcome as judged by parents, teachers, and the clinician.

Publications:

Achenbach, T. M. Assessing youth characteristics. In A. J. McSweeney, W. J. Fremouw, and R. P. Hawkins (Eds), Practical program evaluation for youth treatment. Springfield, Ill., Thomas, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00263-04 LD

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Behavioral Problems and Competencies of Clinic-referred and Nonreferred Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. Thomas M. Achenbach Research Psychologist LDP NIMH

COOPERATING UNITS (if any)

25 Child Guidance Clinics and other mental health agencies

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.25	.45

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to compare the behavioral problems and competencies reported for clinic-referred and nonreferred (normal) children, a survey of 1300 randomly selected parents in the Washington, D.C. area was conducted. Home interviewers administered the Child Behavior Checklist to parents of 50 boys and 50 girls at each age 4 through 16. The results have been compared with parental reports of problems and competencies for clinically referred children matched to the nonreferred children for age, sex, socioeconomic status, and race in order to provide a unique epidemiological baseline for research on child psychopathology and the effectiveness of various treatments.

Project Description:

Although there have been a few normative studies of behavior problems in nonclinical groups of children and other studies of problems in clinical groups, there have been no systematic comparisons of problems and competencies reported for broad samples of clinically-referred and nonreferred children using the same instrument during the same period of time. Comparisons of this sort are badly needed in order to determine what kind of problems and competencies typically differentiate "normal" children from those who are recognized as being disturbed enough to warrant professional attention. Because types of problems and competencies are likely to differ with the age, sex, race, and socioeconomic status of the child, the study is designed to compare referred and nonreferred children matched for these variables at each chronological age from 4 through 16.

The data on the referred children have been collected with the Child Behavior Checklist administered in some 25 child mental health settings in the course of developing the Child Behavior Profile described in the Annual Report on Project No. Z01 MH 00244-05 LDP. The data on the nonreferred children were collected by selecting addresses at random within census tracts stratified so as to obtain a distribution similar to the distribution of clinic cases with respect to socioeconomic status and race. Letters describing the survey were mailed to the selected addresses, and recipients were asked to return a postcard if they had no children aged 4 through 16 living with them. An interviewer then went to each address from which no postcard was returned. If a parent consented to be interviewed and if more than one child between the ages of 4 and 16 resided there, a random number table was used to select the child to be described on the Child Behavior Checklist, excluding any who had received mental health services within the previous year.

The scores obtained by the nonreferred children on most behavior problem and social competence items of the Child Behavior Checklist have found to differ at highly significant levels from those of clinically-referred children within each age and socioeconomic group. Although lower socioeconomic parents reported more problems and lower competence on some items than did upper socioeconomic parents, these differences were far smaller than the referred versus nonreferred differences across socioeconomic strata. Racial differences were minimal within referred and nonreferred samples. Separate comparisons for each sex show highly significant differences between referred and nonreferred children on most items, but the problems that best discriminate the groups vary with the age and sex of the children. These data provide a unique epidemiological baseline for further research on child psychopathology and on the effectiveness of various treatment approaches in bringing a child's behavior into the normal range for his or her age.

This is a final report.

Publications:

Achenbach, T.M. Methods in Clinical research. Yearbook of the National Society for the study of Education, 1980, Part I, 253-268.

Achenbach, T.M. and Edelbrock, C.S. Behavioral problems and competencies reported by parents of normal and disturbed children aged 4 through 16. Monogr. Soc. Res. Child Dev., in press.

Achenbach, T.M. What is child psychiatric epidemiology the epidemiology of? In F. Earls (Ed): Psychosocial epidemiologic studies of children. New York: Neale Watson Academic Publishers, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00265-04 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Chronic Malnutrition and Child Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David E. Barrett	Research Psychologist	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)
Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.65	PROFESSIONAL: .60	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is an investigation of the effects of chronic moderate undernutriton on the social, emotional, and cognitive development of children. Six- to eight-year-old children identified as having a history of malnutrition (protein-calorie deficiency) are compared with children who have had more nearly adequate diets. The children are from vilages that participated in a longitudinal study from which there are extensive data on food intake, health, growth, and earlier cognitive development. The children are all of very similar social and economic backgrounds. Anthropometry and health and nutritional history are used to assess nutritional status. Standard tests and observations in controlled and natural settings are used in investigating cognitive, sensorimotor, and attentional capacities, and social and emotional behavior. Preliminary findings indicate diet calorie intake from birth to 5 years is significantly related to the child's level of social activity. Children Children identified as passive and withdrawn in social interaction had significantly lower diet calorie intakes than those who were socially active. Analyses are currently being conducted examining relations between several composite variables related to nutritonal history (see MH 02138) and indicies of cognitive functioning, sensorimotor abilities, and social interaction characteristics.

Project Description:

This is an investigation of the effects of chronic moderate undernutrition on the social, emotional, and cognitive development of children. Six- to eight-year-old children identified as having a history of malnutriton (protein-calorie deficiency) are compared with children who have had more nearly adequate diets. The children are from villages in Guatemala that participated in a longitudinal study from which there are extensive data on food intake, health, growth, and earlier cognitive development. The children are all of very similar social and economic backgrounds. Anthropometry and health and nutritional history are used to assess nutritional status.

Psychological assessments were made (a) on standard individual tests, and (b) in observations of children in small play groups. The group activities were programmed to permit assessment of a range of responses. Assessments were made on (1) sensorimotor and cognitive variables: attention and information processing, impulse control, reasoning and problem solving; and (2) social-emotional variables: motivation, work efficiency, emotional qualities, attention, and processing of social cues. Field observations were made of the child's home and village environment, and performance and reputation in school were assessed.

The procedures in this project and project MH 02138 are in general parallel, with adaptations and additions appropriate to the two cultures. Data collection has been completed. Data reduction and analysis are underway.

Early analyses indicate the following: (1) Child calorie intake from birth to 5 years is a significant predictor of children's level of social activity and interaction at age 6-8. (2) Child morbidity (ill/days at risk for being ill) during the first five years of life is a significant predictor of children's social activity (negative relationship). (3) Child diet in calories obtained from mother's recall is not related to current height or weight, while child supplement calories (INCAP past intervention) is significantly related to current height and weight. Conversely, child supplement in calories is not related to social activity (vs. passivity) at age 6-8. The findings suggest that diet calories as assessed by a mother recall surveys may represent, to some degree, the mother's attention to and perhaps stimulation of the child. Boys have more diet calories than girls, however, sex differences do not account for the relationship between diet calories and social activity.

Addendum

This project was carried out by the Laboratory staff in collaboration with INCAP. A contract of $25,000 from November 1978 to November 1979 was used to train personnel from local areas and to support them for the collection of data. A member of the Labortory staff was involved in the field work.

Publications:

Barrett, D. E.: A naturalistic study of sex differences in children's aggression. Merrill-Palmer Quart., 25, 193-203, 1979.

Barrett, D. E.: Relations between aggressive and prosocial behaviors in children. J. of Genet. Psychol. 134, 317-318, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00266-04 LDP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Newborn Interaction with Mothers and Nonmothers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob L. Gewirtz	Guest Worker	LDP NIMH
Other:	Albert R. Hollenbeck	Guest Worker	LDP NIMH
	S. Lawson Sebris	Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Alexandria Hospital, Alexandria, Virginia
Holy Cross Hospital, Silver Spring, Maryland

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10		.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation included three related studies: (1) the effects of 2 types of contact (skin-to-skin or cradling) and 2 durations of contact (15 or 60 min.) between a mother and her newborn infant in the first post-partum hour on the mother's attachment to her infant, mother and infant behaviors during observed interaction, and the infant's behavioral development; (2) the effects of 2 durations of contact (5 or 60 min.) between a father and his infant born by cesarean section in the first 1-1/2 hours post-partum on the father's attachment to his infant, interaction of the father and infant, mother's interaction with the infant, and the infant's behavioral development; and (3) the effects of a program of intense interaction between a nursing student and an infant on the student's attachment to the infant and the infant's behavioral development. Preliminary analyses of Study 1 have found differnces in both mother and infant behaviors as a result of the duration and type of contact in the first hour after birth.

Project Description

Recent work has drawn attention to the fact that skin-to-skin post-partum contact between neonate and mother in the first hour (deChateau, 1976; Hales et al., 1977) and extended contact of mother and infant during the first three days (Klaus, Jerauld, Kreger, McAlpine, Steffa, & Kennel, 1972) could result in maternal behaviors denoting attachment to the infant, both in the newborn and in subsequent periods. This research has the primary purpose of replicating and extending studies of the possible effects of immediate post-partum adult-infant contact on the development of the adult's attachment to the infant and the behavioral development of the infant.

This research involved 3 related studies of post-partum contact conditions between an infant and either its mother, father, or a nursing student. (1) The effects of 2 types of contact (skin-to-skin or cradling) and 2 durations of contact (15 or 60 min.) between mother and her newborn infant in the first post-partum hour on the mother's attachment to her infant, mother and infant behaviors during observed interactions, and the infant's behavioral development; (2) the effects of 2 durations of contact (5 or 60 min.) between a father and his infant born by cesarean section in the first 1-1/2 hours post-partum on the father's attachment to his infant, interaction of the father and infant, mother's interaction with the infant, and the infant's behavioral development: and (3) the effects of a program of intense interaction between a nursing student and an infant on the student's attachment to the infant and the infant's behavioral development. Behaviors which may denote attachment were observed during subsequent adult-infant interactions. Infant subjects for all studies were normal, healthy, fullterm newborns from white, intact middle-class families.

Preliminary analyses have been completed on Study 1 and Study 2. In Study 1, effects have been found in both mother and infant behaviors during observed mother-infant interactions. These effects have been found for both the duration of post-partum recovery room contact (15 or 60 minutes) and the type of presentation (cradling or skin-to-skin) during that contact. As an example of a result pattern detected to date, mothers who had the initial skin-to-skin contact with the infant were rated at 28 days as showing closer physical involvement with their infants, more satisfaction with their infants' characteristics, and more appropriate behavior in connection with their infants' feeding.

In Study 2, no paternal differences were found, nor were there differences between paternal and maternal behaviors in interaction with their infant. No delivery mode differences have been found in maternal behaviors in interaction with their infants; however, several differences in infant behaviors been found. Cesarean-sectioned infants exhibited higher rates of "en-face" looking and mouthing than did vaginally delivered infants. In addition, several differences were detected between infants in the two delivery groups on several physiological measures of the Brazelton Scale.

This project has been discontinued because the principal and other investigators are no longer at NIMH. Analysis of the studies are continuing elsewhere.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00269-03 LDP

PERIOD COVERED October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Assessment of the Outcome of Child Psychiatric Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)

Clifford Beers Guidance Clinic, New Haven, Connecticut

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER: .27

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose was to assess relations between the patterns of problems and competencies displayed by children referred for guidance clinic treatment and the outcome of two widely offered treatment modalities, namely, individual psychotherapy and conjoint family therapy. The subjects were applicants to a guidance clinic who manifested behavior patterns corresponding to several preselected types and whose therapists judged individual psychotherapy and conjoint family therapy to be equally good treatment modalities for them. If the child and family consented, the therapist administered either psychotherapy or family therapy, as determined by the research design. The course and length of therapy depended on the child's progress and the therapist's judgment, but an independent evaluator obtained ratings from the parents and the child's teacher at the beginning of treatment and again at 6 and 12 months after intake.

PHS-6040
(Rev. 10-76)

Project Description:

Despite the large number of children in need of mental health services, there have been very few controlled studies of the effectiveness of such services. Even the best of the few existing studies suffer from a fundamental defect: They have assessed the effectiveness of treatment indiscriminately across heterogeneous groups of children. Despite the fact that no single approach to treatment is likely to be uniformly optimal with all kinds of children from all kinds of backgrounds manifesting all kinds of problems, these studies have not been designed to determine what kind of treatment is most beneficial for which children.

The purpose of the present study was to perform a controlled evaluation of the effectiveness of different outpatient treatment modalities with children who are grouped according to demographic characteristics (age, race, sex, socioeconomic status) and according to patterns of behavioral problems and competencies scored on the Child Behavior Profile. The Profile has been developed by Dr. Achenbach as a standardized instrument for describing children's problems and competencies, for identifying overall behavior patterns, and for measuring behavioral change. Cluster analysis of Profile patterns has made it possible to identify a limited number of "types" that characterize the vast majority of children seen in outpatient psychiatric facilities. This typology of Profile patterns provides a basis for classifying children in order to determine which ones benefit most from which type of treatment. In our study, applicants to Clifford Beers Guidance Clinic who were matched with respect to age, sex, socioeconomic status, race, and Child Behavior Profile type received one of two treatment conditions: individual psychotherapy or conjoint family therapy. The focus was not on which therapy is the better of the two but on differences in outcomes for different types of children who have received the two different types of treatment. Thus, several highly informative findings were possible. For example, it might be found that certain types of children do well under one treatment and poorly under the other. This would provide a basis for more precise prescription of treatment based on child characteristics. Another possible finding is that certain types of children do well under both treatments and other types do poorly under both treatments. This would indicate the need for trying a wider range of treatment modalities with the latter group or for developing new approaches if no existing approach is found to be effective with these children. Whatever the outcome for each group, the fact that children who are similar with respect to Profile type, age, sex, race, and socioeconomic status receive different treatments according to a systematic research design should permit a far stronger basis for inferring the effects of such treatment with each type of child than is possible without controlled assignment to treatment conditions.

The initial phase of the study included only boys aged 6 through 11 having any one of four behavior Profile types we have identified for this group. The reason for beginning with this group is that they are by far the most numerous clients of child guidance clinics. Because the number of participating therapists was limited, and because each therapist had continuing responsibility for the children who became part of the study, the chances of obtaining at least 10 subjects in each of the 4 (Profile types) x 2 (treatment types) = 8 cells of the research design were best with this group.

Boys aged 6 through 11 who had one of the four target Behavior Profile types and whose therapist judged individual psychotherapy and conjoint family therapy to be equally appropriate for them were assigned to the two treatment conditions in such a way as to produce distributions that were as similar as possible with respect to age, race, and socioeconomic status. This was done by having an independent evaluator score the child's intake Profile after the therapist's clinical evaluation but before the therapist met again with the family to discuss treatment. If the Profile corresponded to one of the four target types, the independent evaluator determined which of the two treatments should be provided as follows: For the first 10 cases having each Profile type, the evaluator alternated treatment assignments so that the first case having either of two Profile types received individual therapy, whereas the first case having either of the other two Profile types received conjoint therapy. Thereafter, the second case of each type received the treatment that the first case did not receive, and the alternation of assignments to treatment continued until five cases having a particular Profile type were assigned to each treatment. Because she maintained a record of the age, race, and socio-economic status of each case, the independent evaluator was able to identify and correct any important differences in the distribution of these variables between cells of the research design.

If a family consented to participate in the study, the data from the Child Behavior Checklist they filled out as a routine part of Beers' clinical evaluation, plus a Teacher's Report obtained with the parents' permission and the therapist's ratings on family functioning, provided the baseline against which behavioral change was measured. Since all the participating therapists were skilled in both types of therapy, the same therapist treated the child regardless of which therapy was selected according to the treatment design. The length of treatment was determined by the therapist's clinical judgment and the family's motivation rather than by the research design. Whenever termination occurred, the therapist rated the child and family on various aspects of functioning.

The major focus of the statistical evaluation was to be on changes in the behavioral problems and competencies reported by parents, therapists, and teachers from intake until termination and until the 6- and 12-month follow-ups for children of each Profile type receiving each type of treatment. One dimension of analysis was to be a comparison of outcomes for children having a particular Profile type but receiving the two different treatment modalities. This was to be done in order to determine whether one type of treatment has been more successful than the other type of treatment with children having a particular Profile type. A second dimension of analysis was to be a comparison of children having different Profile types in order to determine whether profile pattern was consistently related to outcome, regardless of type of treatment. The relations between the therapists' intake ratings and the outcome measures were also to be assessed in order to determine whether they predict outcome independently of or in conjunction with Profile type and treatment modality.

The data obtained in Project Z01 MH 00269-03 are being analyzed in conjunction with earlier follow-up data from the same clinic (see Project 00246-05).

This is a final report.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00270-03 LDP

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Identification and Validation of Syndromes of Child Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.: Thomas M. Achenbach Research Psychologist LDP NIMH

COOPERATING UNITS (if any)

25 Child Guidance Clinics and other Mental Health Agencies

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to identify and validate syndromes of child psychopathology, we have conducted a literature review of all efforts to derive such syndromes empirically, have made cross comparisons among studies to identify replicated syndromes, and have performed cluster analyses of Child Behavior Profiles in multiple clinical samples. Clusters found to replicate across samples have been used to classify children in order to identify demographic, adaptive, and prognostic correlates of the syndromes thus represented.

PHS-6040
(Rev. 10-76)

Project Description:

The lack of an accepted taxonomy for child psychopathology has stimulted many efforts to identify syndromes of behavior problems through multivariate statistical techniques. However, the diversity of subject samples, raters, data collection instruments, techniques of analysis, and methodological flaws made it necessary to do a systematic review and cross-comparison of findings in order to determine whether there is consistent evidence for any syndrome, despite the methodological differences among studies. We found that a distinction between broad-band and narrow-band syndromes seemed warranted because many syndromes derived from first-order factor analyses could be subsumed by a few second-order factors. Broad-band "undercontrolled" and "overcontrolled" syndromes and narrow-band aggressive, delinquent, hyperactive, schizoid, anxious, depressed, somatic, and withdrawn syndromes were found in diverse samples of disturbed children. Two other broad-band and six other narrow-band syndromes were found in a few studies. Test-retest reliabilities and stabilities of syndrome scores were more adequate than interrater reliabilities, which were inversely related to the degree of disparity between types of raters and situations in which they saw subjects. Cross-instrument and cross-population consistencies corroborated some empirically derived syndromes, but the lack of independent criteria for categorizing disturbed children makes it difficult to establish criterion-referenced validity.

Because most efforts to derive syndromes empirically have not been extended to categorization of individual children and the few previous efforts have been limited primarily to the broad-band undercontrolled versus overcontrolled dichotomy, we have applied cluster analysis to Child Behavior Profiles in order to obtain a more differentiated taxonomy. Profile patterns that are replicated through cluster analyses of multiple clinical samples have been used to classify children. Differences among the children in demographic characteristics, social competencies, psychiatric diagnoses and the outcomes of clinical contacts have been assessed in order to determine the correlates of the cluster-based taxonomy.

This is a final report.

Publications:

Achenbach, T. M. Assessment of childhood behavior disorders. In Woody, R.: Encyclopedia of Clinical Assessment. San Francisco: Jossey-Bass, 1980.

Achenbach, T. M. Empirical approaches to the classification of child psychopathology. In Lachenmeyer, J. R. and Gibbs, M. S. (Eds.): Psychology of the Abnormal Child. New York: Gardner, in press.

Edelbrock, C. S. and Achenbach, T. M. A typology of Child Behavior Profile patterns: Distribution and correlates for disturbed children aged 6-16. J. Abnorm. Child Psychol.: in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02131-03 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Conditions Influencing Interaction between Parents, Nonparents, and Neonates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob L. Gewirtz, Ph.D.	Guest Worker	LDP NIMH
OTHER:	Albert R. Hollenbeck, Ph.D.	Guest Worker	LDP NIMH

COOPERATING UNITS (if any)
Alexandria Hospital, Alexandria, Virginia
Columbia Hospital for Women, Washington, D. C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.08	.02

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study focuses on the influence of infant characteristics on parent behavior. Specifically, effects on mother's behavior of the attribution to the infant of particular appearance or behavioral characteristics are examined. Three conditions are studied: (1) Mothers are asked to provide infants with "love" or "comfort" --both abstract examples of nurturance. Analyses are made of the mother-infant interaction to which each abstraction reduces. (How these patterns of interaction relate to situational and demographic variables is also examined.) (2) Similar observations are made of hospital nurses when they interact with infants arbitrarily labeled male or female. (3) Following a nominal examination of the infant, the investigator makes a statement to the mother emphasizing a transient infant characteristic. Parental and infant responses (e.g., hugs, smiles, verbalizations, kisses, squeezes) are assessed.

PHS-6040
(Rev. 10-76)

Project Description

This study focuses on the influence of infant characteristics on parent behavior. Specifically, effects on mother's behavior of the attribution to the infant of particular appearance or behavior characteristics are examined in three related studies. In the first study mothers' response patterns when they are instructed on how to interact with infants were investigated. Mothers are asked to provide infants with "love" or "comfort"--both abstract examples of nurturance. (Within-subject control observations are made by having each mother "get to know your infant".) Nurturance has been an important variable in descriptions of infant development; yet, terms such as "love" and "comfort" are ambiguous as well as idiosyncratically used. We are examining nurturance in a more direct fashion. From our observations, we hope to develop a taxonomy of adult behaviors to infants that define abstract terms like "love" and "comfort".

An hypothesis about adult behavior that persists in the child-rearing literature is that adults respond differentially to children depending on the child's sex, and that this differential treatment, in turn, explains later differences between the behaviors of boys and girls. The aim of our second study is to compare the response patterns of adults to infants, when those infants are arbitrarily labeled male or female. For half of the cases, hospital nurses are told the true sex for a stranger infant with whom they are to interact; for the other half of the cases, the nurses are told the incorrect sex.

Parent behavior to infants also may be influenced by messages communicated to parents by persons having contact with their baby. The aim of the third study is to investigate how adult-infant interaction may be influenced by an attribution to the infant of particular transient behavioral characteristics. Thus, mother-child interaction is first observed under standard conditions. Then the staff member remarks to the mother that the infant is (a) "more active" or (b) "less active" than he or she was during a time when the infant had been alone with the staff member. Observations of resulting mother-infant interaction are made and comparisons are made between patterns of interaction observed before and following the observer's comment.

Work on data collection has been completed for study one (48 subjects) and three (52 subjects) and is nearing completion for study two (14 out of 24 subjects). Data reduction and analyses are underway.

This project has been discontinued because principal and other investigator are no longer at NIMH. This is a final report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02132-03 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Children's Misdeeds, Parental Discipline, and Children's Compliance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.20	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Effects of children's misbehaviors on parents' disciplinary methods were studied. A specific purpose was to explore antecedents of physical punishment and love withdrawal, as well as antecedents of reasoned discipline practices. Twenty-four mothers of one-to-two-year-old children were trained to report incidents of children's interactions, from which sequences of misbehaviors, discipline, and subsequent compliance or noncompliance were extracted. Misbehaviors were categorized as harm to persons, harm to property, and "lapses" in self-control. Forms of discipline were reasoning, teaching, dramatization of wrongdoing, verbal prohibitions, physical punishment, physical restraint, and love withdrawal. Half of mothers' initial disciplines were verbal prohibitions. Explanations and physical restraint were also common control methods. Following children's noncompliance to discipline, mothers used a wider range of discipline practices. Transgressions against persons were likely to be associated with psychologically oriented reasoned forms of discipline. Destruction of property and lapses in self-control were likely to be associated with parental techniques that incorporated overt displays of power and exertion of strong controls.

Project Description:

Research on children's personality development in relation to caretakers' socialization practices has been based largely upon the assumption that the parent exerts a predominant influence on the child. More recent conceptualizations have emphasized the child's active contributions to the socialization process as well. The purpose of this research is to study the interaction of children's misbehaviors with parents' disciplinary practices. What determines, for example, the conditions under which a parent will hit a child, explain, scold, or isolate the child following transgression? Of special interest are the antecedents of physical punishment and love withdrawal, since these two discipline techniques, in the extreme, constitute conditions of child abuse and neglect, as well as the antecedents of more "reasoned" forms of discipline. Here, we examine whether different forms of child misdemeanors, behaviors that parents often perceive as undesirable and requiring intervention, lead predictably to different types of discipline.

The sample is 24 middle class mothers and their one-to-two-year-old children. Data were obtained over a nine month period. Mothers were taught to observe and report incidents of children's interactions, involving emotions, from which sequences of misbehaviors, discipline and compliance or noncompliance could be extracted. Ratings were also made by investigators of mother and child during home visits. When these ratings were compared with mothers' reports there were significant similarities between the two data sources. Coder reliabilities (percent agreements) on mothers' reports of categories of transgressions and disciplines ranged from 66% to 92% (average = 80%). The children were found to engage in many behaviors that required disciplinary interventions. Three general classifications of misbehavior were used: harm to persons, harm to property, and problems with impulse control. Mothers' techniques of discipline were coded as explanation, guidance, verbal prohibitions, physical punishment, physical restraint, and withdrawal of love. Mothers' dramatizations of distress or highlighting of misdeeds were also frequent reactions to misbehaviors. About half of mothers' initial methods were verbal prohibitions; following children's noncompliance, mothers used a wider range of disciplines and used them differentially, depending upon the form of misbehavior. Children's transgressions against persons tended to be followed by (or associated with) psychologically oriented forms of discipline (e.g., reasoning and dramatization of distress). Misbehaviors involving destruction of property and lapses in self-control were associated with parental techniques that incorporated displays of power and the exertion of strong controls (e.g., physical punishment, restraint, and love withdrawal), though other methods of discipline were seen as well. These findings have implications (a) for interpretation of direction of influence in socialization research and (b) for understanding some of the conditions that lead parents to lose control in the handling of children's misbehaviors.

A manuscript has been submitted for consideration for publication and a second manuscript is in preparation.

Publications:

Chapman, M. Listening to reason: Children's attentiveness and parental discipline. Merrill-Palmer Quart., 25, 251-263, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02133-03 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Stability and Change in Children's Responsiveness to Others' Emotions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.29	.25	.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is concerned with developmental stability and change in children's responses to emotional distress in other persons. This is a follow-up at ages 6 to 7 of children who were studied in the second and third years of life (MH 00242). Data for both periods are detailed descriptive accounts of responses to another person's distress. Naturally-occurring and simulated distress situations were the basis of these accounts. Individually patterned ways of responding were apparent by the end of the second year of life. Considerable consistency from the early to the later age period was found in individual children's modes of responding to others' emotions.

Project Description

This study is concerned with stability and change in children's affective responding over time. Exposure to the emotions and feelings of other persons elicits diverse reactions from young children. In an earlier study (MH 00242) these responses were investigated in boys and girls in the second and third years of life (N = 24). Based on extensive behavior samples during this time, it was found that by the end of the second year of life, individual children's responding was distinctly patterned. The present study is a follow-up of these children, now between 6 and 7 years of age, to investigate the stability and change in their modes of responses over the course of development.

The data for both time periods are detailed narrative accounts of children's behaviors in situations in which the child is an observer of another person's distress. (See earlier reports for description of method, MH 00242). Data covered 9 months of the child's development in the early years and 3 months at ages 6 and 7. At the older ages, standard laboratory assessments were also made of children's interaction with a person in a minor distress, and of children's reasoning about other's emotions.

To assess the nature of consistency of behavior across developmental periods, responding at early and later ages was analyzed (independently) for intensity of affective arousal, orientation of approach or avoidance in circumstances of distress, inferential abilities, and distinctive modes of expression of affect and action regarding others' distress. Considerable consistency exists with regard to the nature of affective arousal and mode of reacting to the person in distress. The evidence in stability over time derives from both the naturalistic data and the laboratory assessments.

A paper has been submitted for publication, and a second is in preparation.

This is a final report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02134-03

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Developmental Changes in the Understanding of Pictures Representing the Production of Sound

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sarah L. Friedman	Guest Worker	LDP NIMH

COOPERATING UNITS (if any)
University of Wisconsin, Madison, Wisconsin 53706

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .05	PROFESSIONAL: .05	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this investigation are examined age changes in the ability to interpret correctly pictorial representations of sound production. Sound cannot be captured in a three dimensional picture; however, the production of sound can be represented by various techniques: (1) Postures. Some postures suggest that sound is being produced; for example, a person may have his mouth open and lean toward the listener. (2) Context. Musical instruments, tools, and other objects are understood to make sound when they are held or moved in certain ways. (3) Arbitrary methods are used to suggest sound production. These methods include balloons containing words, lines, or musical notes drawn next to the source of sound. Pictures using the three techniques for representing sound and matched pictures without indications of sound will be shown to subjects between 2 and 20 years of age. It is expected that there will be an interaction between age and the pictorial technique used when the correctness of interpretations is examined.

Dr. Friedman is no longer with NIMH and the project is terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02135-03 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Emotional Development in Children of Depressed Parents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)
Laboratory of Biological Psychiatry, NIMH
Laboratory of Psychobiology, NIMH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.20	.10

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The emotional development of children in families in which one of the parents is suffering from an affective disorder is examined. This study is an extension of a program of research on children's encounters with the emotions of others (Z01 MH 00242-06 LDP). Families are those in which a parent has a history of depression and/or a history of bipolar depression. Children from families with no known psychopathology are also studied. Families begin the study when children are 10-12 months old and are followed longitudinally. Observations of children's responses to emotional events in the familial environment are obtained in the form of narrative accounts from parents. Also, observations of mother-child interaction in the home are videotaped, as are children's responses to structured situations in which mothers portray different specified emotions. Data collection is in progress. The purpose of focusing on young children's responses to emotions, both to the transitory distresses and the more enduring distress states of others, is to learn more about processes by which children develop and maintain adaptive and maladaptive patterns of coping with emotions.

Project Description:

The focus of this research is the emotional development of children in families in which one of the parents is suffering from an affective disorder. These studies are an extension of a program of research in which children's encounters with the emotions of others are examined. The previous studies have been of children in families that are free of severe psychopathology, and of children in the first two years of life. Emphasis has been on discrete emotional events, with little attention to a systematic conceptualization and sampling of the affective environment of the family. In the present study children of similar ages are studied, but with greater attention to (a) the chronic aspects of the emotional encounters, and (b) the development of additional methodological approaches for measuring children's responses to emotions of others.

Mothers of 10-12-month-old children are trained to report, daily, significant events of positive and negative emotions occurring in the natural environment (see Annual Report Z01 MH 00242-06 LDP for description of reporting procedures.) To assess affective communication patterns further, child and mother are videotaped in a session in the home when the child is 18 and 21 months of age. In some portions of the session, the mother is asked to vary (according to a script) her emotion expression, e.g., sorrow about a sad story in the newspaper, pain from bumping into a piece of furniture, choking over a drink of water. Later the mother is asked (a) to engage in 5 minutes of spontaneous play with the child; (b) then to assume for 2 minutes a "neutral", expressionless face, responding to the child's requests but not initiating interaction, and (c) then to resume play. The purpose of exploring mother's affectless expression is to study, generally, how children respond to maternal emotional unavailability; also, specifically, to examine individual differences in children's interpretations of depressed affect. Children's responses to photographs of positive and negative emotions in infants are measured, as well as how mothers use these photographs to teach their children about the emotions of others. Twenty-six families are studied. Twenty are normal families. In six families one of the parents is diagnosed as having a bipolar affective disorder and in most of these instances, the spouse is also diagnosed as depressed. By exploring children's responses to others' transitory and enduring affective expressions in relation to the affective characteristics of the rearing environments, we are attempting to learn more about the motivating factors that have an early influence on interpersonal orientations toward others' emotions.

Data collection is still in progress; additional families in which there is significant parental depression are being recruited.

This work was described in a symposium-workshop on infant reactions to emotional signals at the International Conference on Infant Studies, April, 1980, in which a videotaped presentation of empathy and sympathy in children in the second year of life was made.

Publications:

Zahn-Waxler, C. and Yarrow, M. R. Research strategies for studying prosocial behavior in children. In Eisenberg-Berg, N. (Ed.) The Development of Prosocial Behavior. New York: Academic Press, Inc. In press for 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02136-03 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Prosocial Interaction Among Physically Handicapped and Nonhandicapped Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Karabelle A. L. Pizzigati	Guest Worker	LDP NIMH
OTHER: Gale Inoff	Psychologist	LDP NIMH

COOPERATING UNITS (if any)
Montgomery County Public Schools

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.15	.10	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recent legislation indicates that there will be greater integration (i.e., mainstreaming) of handicapped children. The resulting social adjustment of handicapped and nonhandicapped children to each other is a major concern. The purpose of this project was: (1) to compare, in terms of their social, play, and affective behaviors, handicapped and nonhandicapped children, (2) to investigate the effects of being from a mainstreamed school, and (3) to study the effects of having a handicapped child in a play group of nonhandicapped children. Experimenters observed and coded the behaviors of orthopedically handicapped and nonhandicapped children ages 5-9 years in small group play sessions. Nonhandicapped children were found to be more often involved in the social exchanges and group activities than were the handicapped children. Having been to a mainstreamed school positively affected the way nonhandicapped children interacted with a handicapped child. Other than a statistically nonsignificant tendency for the presence of a handicapped child to inhibit the group, groups with and groups without a handicapped child were highly similar in those social, play, and affective variables assessed.

PHS-6040
(Rev. 10-76)

Project Description

In the past, handicapped and nonhandicapped children have been segregated in many settings. Recent legislation, however, indicates that there will be increasing integration (i.e., mainstreaming) of handicapped children, particularly in public school settings. The resulting social adjustment of handicapped and nonhandicapped children is a major concern.

The purpose of this project was: (1) to compare, in terms of their social play and affective behaviors, handicapped and nonhandicapped children, (2) to investigate the effects of being from a mainstreamed school, and (3) to study the effects of having a handicapped child in a play group of nonhandicapped children.

Children ages 5-9 were observed in groups of 6. Twenty groups were seen. Group composition consisted of either 1 handicapped child and 5 nonhandicapped children or 6 nonhandicapped children. Of the groups having a handicapped child, some of the groups had nonhandicapped children from a mainstreamed school and some had nonhandicapped children from a non-mainstreamed school. The handicapped children were orthopedically handicapped.

The children were observed on measures such as social participation, friendly interaction, negative interaction, helping behavior, task involvement, and happy affect. Some measures were assessments of group functioning; others were assessments of individual children within the group.

Comparisons of nonhandicapped and handicapped children indicated that the nonhandicapped children were more often involved in social exchanges and group activities than were the handicapped children. However, for many variables (e.g., happy affect, negative interaction), there were no differences between handicapped and nonhandicapped children.

Comparisons were made between groups of mainstreamed and nonmainstreamed children, each type of group having one handicapped child and five nonhandicapped children. For most variables, no differences were found; for variables assessing interaction between the handicapped and nonhandicapped children (e.g., initiating and responding to the handicapped child, helping the handicapped child), mainstreaming effects were evident. Having been to a mainstreamed school seemed to contribute toward attending to and showing prosocial behaviors to the handicapped child.

Comparisons were made between groups having a handicapped child and groups with only nonhandicapped children. Although there was a slight tendency for the presence of a handicapped child to inhibit the group, in general, the groups were very similar and those differences which were found were not statistically significant.

It also appeared that the degree to which a handicapped child was ignored was related to the degree to which he or she was passive and failed to initiate interactions. Those handicapped children (like nonhandicapped children) who made demands on the group were less likely to be ignored.

A manuscript has been submitted for publication.

This is a final report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02138-02 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Undernutrition and Behavioral Competence in School-age Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Marian Radke-Yarrow	Chief	LDP NIMH
Other: David E. Barrett	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)
Department of Neurosciences, School of Medicine, University of California at San Diego, LaJolla, California 92037

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.45	.45	1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a study of children who have a history of moderate to severe undernutrition and currently have a marginally adequate diet. Cognitive and social competencies, it is hypothesized, are impaired by undernutriton experienced in the early years. Learning disorders and difficulties in social relationships may be derived from these impairments. One hundred and twenty 7-year olds from very low economic Anglo families in Southern California were the subjects. Anthropometric measures of child and family, nutritional and health history and status of mother and child, and blood analyses are the bases for classification. Behavioral assessments included measures of sensorimotor, cognitive, and attentional capabilities, impulse control, affective characteristics, goal-directed and work effective activities, and social interactional characteristics. Data reduction and data analysis are underway.

PHS-6040
(Rev. 10-76)

Project Description:

This is a study of children who have a history of moderate to severe undernutrition and currently have a marginally adequate diet. Cognitive and social competencies, it is hypothesized, are impaired by undernutrition experienced in the early years. Based on animal research and on findings on severe childhood malnutrition, impairments are expected in attentional and cognitive abilites, in motivation and goal-directed behavior, and in emotional control. Learning disorders and difficulties in social relationships may be derived from these impairments.

The children in this study are 120 seven-year-olds, from Anglo families, living in poor urban areas of Southern California. All families were below a poverty income level.

The child's nutritional history and status were determined on the basis of height for age, weight, skinfold, nutritional and health history and status of mother and child, and blood analyses. Based on the combined criteria, children were assigned to one of three groups: (a) small-for-age (tenth percentile or below) with a history of undernutrition, (b) small-for-age (tenth percentile or below) without a history of undernutrition, and (c) average or above height for age and without a history of undernutrition.

The children were tested on the WISC, Bender-Gestalt, Lindamood, and Bruininks- Oseretsky. There were experimental tests of social and emotional behavior. The children were seen also in small groups in two half-day sessions in which there were measures of the social and emotional variables indicated above.

Each family was visited to obtain further data on the home and neighborhood. In visits at the end of the study, the family was given information about the child's physical health and psychological status, and was provided with referrals to professional help in cases of problems.

An attempt has been made to develop nutritional and health "composite variables" representing "risk factors" predictive for later development. These composites are based on risks-by-time (prepregnancy, pregnancy, birth, 0 to 2 years, and 2 years to 7 years) and also, other composites conceptualized risk factors in terms of brain development. Certain composite variables (illness during pregnancy, especially in the third trimester; and child illnesses affecting nutritional status) are significant predictors of poor visual-motor integration.

The composite risk factor, child health 2 to 7 years was signficantly correlated with the Performance scale of the WISC. Composites of child health (0 to 2 years, and 2 years to 7 years) were significantly correlated with WISC Verbal score. The risk composites referring to child health (birth to 2 years and 2 years to 7 years) were both significantly correlated with two scales of sensorimotor abilities, strength, and upper-limb coordination. The analyses are in early stages.

Addendum:

This project was carried out off-site. The data collection was performed on a contract that terminated September 30, 1979. This included case finding, screening, nutritional history and status, and the psychological measurements.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02139 - 02 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Social Cognition and Social Behavior in Situations of Emotional Distress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.35	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children's cognitive, affective and behavioral capabilities in coping with the emotional distresses of other persons are investigated. Two sets of information, one of cognitive data and one of affective and behavioral data are obtained on two samples of children: (1) Twenty-two, 5-7 year old children who constitute a longitudinal sample and (2) 60, 4-11 year old children who are part of a cross-sectional research design. The Peabody Test of mental ability, role-taking tests (Flavell, Chandler) and interpretations of incidents of others' emotional distress (portrayed in eight structured stories) are examined in relation to the child's responding to naturalistic and/or simulated distress incidents. Data collection is completed and coding and analyses are under way. Preliminary analyses indicate that some of the cognitive procedures for assessing children's understanding of others' experiences (Flavell role-taking tests) tend seriously to underestimate young children's social-inferential capabilities.

PHS-6040
(Rev. 10-76)

Project Description:

Children's capabilities in coping with the emotional distress of other persons are investigated. Although role-taking abilities and prosocial behavior have been studied extensively as separate issues, their investigation together has been mainly in laboratory studies in which very limited samples of role-taking and prosocial behavior have been examined in relation to one another. Therefore, there is little basis on which to develop understanding of the interdependencies of these processes. The focus of this research is on these interdependencies, making use of substantial response sampling, covering an extended period of naturalistic data collection and a 1½ hour laboratory session.

The Peabody Picture Vocabulary Test of verbal IQ, two perspective-taking tests (Flavell, Chandler), verbal interpretations of eight story distress incidents, and responses to simulated distress incidents in the laboratory were obtained for two samples of children: (a) 5 girls and 5 boys at ages 4, 5, 6, 7, 11, and 12 (Total $N = 60$) and (b) twenty-two girls and boys, ages 5-7, for whom maternal reports of their responses to naturally occurring distress incidents in the home were also available. Sample a provides a cross-sectional examination of developmental age changes in perspective-taking, empathy, and prosocial behavior, while sample b makes possible a comparison of laboratory measures with responses to distress in naturally occurring circumstances.

Data collection has been completed; coding and analyses are under way. Preliminary analyses indicate that in the naturalistic data, by 5-7 years the capabilities of children for making sophisticated inferences about others' emotional states are well documented by their affect, action, and words. In the laboratory setting, the same children, when asked to interpret the thoughts and motives of children witnessing distresses, showed levels of inferential abilities consistent with findings from the naturalistic data. In the same laboratory session, when these children were given Flavell's role-taking test (7-picture story), a frequently used index of perspective-taking ability, eighty percent failed this test. Thus, some widely used indices of perspective-taking may seriously underestimate young children's social-inferential abilities.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02140-02 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Interrelations of Early Cognitive Development and Empathic Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Chapman	Staff Fellow	LDP NIMH
Other:	Carolyn Z. Waxler	Research Psychologist	LDP NIMH
	Marian R. Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .45	PROFESSIONAL: .30	OTHER: .15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research objective is to examine the development of empathy and prosocial behavior in relation to progressive differentiation of self and other in the second year of life. Data on children's empathic capabilities are taken from mothers' systematic observations of their children's responses to naturally occurring emotional incidents in the home environment. Understanding of self and other is assessed by a Self Recognition Scale (children's reactions to their own mirror image) and an Agent Use Scale (understanding of other persons as independent agents in the context of pretend play). An Object Permanence Scale is also administered as a standard measure of cognitive development in the sensorimotor period. Thirty children are studied from 10 months of age until two years. Data collection is under way.

PHS-6040
(Rev. 10-76)

Project Number Z01 MH 02140-02

Project Description:

Empathy, the capacity to share another person's feelings or emotions, is thought to be the primary motivation for prosocial behaviors such as altruism, helping, and cooperation (Bryan, Hoffman, Stotland). It has been further suggested that the development of empathy is related to the progressive differentiation of self and "other" in the second year of life (Hoffman). Clinical evidence suggests that disturbance in differentiation of self and other may result in later pathology (Mahler, Bowen).

The present study focuses on the hypothesized relation between the understanding of self and other and the development of empathy and prosocial behavior. Two scales assessing the understanding of self and other are administered to 30 infants between the ages of 12 and 24 months. One group of 10 is tested every two months. Another group of 10 is tested at 12, 18, and 24 months. The third group consists of children, one of whose parents has a bipolar affective illness. Tests are administered in the infants' homes. Empathic, prosocial, and emotional behaviors are assessed from systematic maternal observations as used by the investigators in previous studies (Z01 MH 00242-05).

In the Agent Use Scale (Watson & Fischer), children are observed in play to determine whether or not they imitate actions involving self-other relations previously demonstrated by the experimenter. It is predicted that the development of children's understanding of other persons' actions as independent from their own will parallel the development of the understanding that other persons' distress is independent of their own.

In the Self-Recognition Scale (Bertenthal & Fischer), the infant is given a series of self-recognition tasks in front of a full length mirror. It is predicted that the development of self-recognition will also parallel the development of empathic and prosocial behavior.

An Object Permanence Scale (Uzgiris & Hunt) is also given as a standard measure of sensorimotor development. It is predicted that scores on this scale, designed to measure infants' understanding of the physical world, will not be as closely related to the development of empathy as scores on the Agent Use and Self-Recognition Scales, which are intended to assess social understanding. The inclusion of a subsample of infants with bipolar depressed parents will enable the investigators to determine how an infant's cognitive development is affected by a deviant affective environment.

Data collection has been completed on approximately half the sample and continues for the remaining half.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02141-02 LDP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)
Assessing the Progress of Children in Residential and Day Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)
Hillcrest Children's Center, Washington, D. C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.10	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relations among ratings--made by parents, teachers, child care workers, and an independent observer--of the behavioral problems and competencies of children in residential and day psychiatric treatment; (2) to determine the relations between children's initial behavior patterns as reported by these sources and the children's progress in treatment. The initial ratings were made by parents, Hillcrest staff, and the independent observer at the beginning of the academic year and again in the late spring. Relations between initial behavior patterns--derived from ratings by the various sources--and children's progress--as measured by the follow-up ratings and indices of academic functioning--are being compared with similar data on out-patient samples to determine the prognostic validity and generality of the initial behavior patterns.

PHS-6040
(Rev. 10-76)

Project Description:

Because judgments of children's behavioral problems and competencies are inevitably influenced by the conditions under which the children are observed and by the observers' relationships to the children, it is essential to identify areas of agreement and disagreement among observers and to determine the best sources of observations for assessing psychopathology, making treatment decisions, and evaluating change in disturbed children.

The purpose of this project is to extend to children in day and residential treatment the methods of assessment we have developed and applied to children treated on an outpatient basis (Project No. Z01 MH 00259). The subjects were 40 children aged 6 to 13 in treatment at Hillcrest Children's Center in Washington, D. C. The rating instruments were versions of the Child Behavior Checklist (Project No. Z01 MH 00244) that have been developed for raters having different relationships to the children (parents, child care workers, teachers, direct observers).

The design of the study entailed obtaining behavioral ratings from the following observers: Ratings on the Child Behavior Checklist from the child's parents if the child lived at home or from child care workers if the child lived at the treatment center; ratings on the Teacher Report Form from the child's teacher and teacher's aide; and direct observational ratings from a trained observer who observed the child for six 10-minute periods in class and six 10-minute periods in recess. During each 10-minute time sample, the observer wrote a narrative description of the child's behavior. From this narrative, she then rated the child on behavior problem items drawn from the Child Behavior Checklist, using 4-step response categories. She also scored on-task behavior at 1-minute intervals within each 10-minute sample.

The ratings were made in the autumn and again six months later in order (a) to determine agreement among the various observers at the beginning of a school year and again at the end of the school year, and (b) to determine which source or combination of sources of ratings best predicts outcomes over the course of the school year. In addition to behavioral ratings, achievement and cognitive measures will be used as indices of improvement.

It is expected that comparison of changes for children differing in initial behavioral patterns will provide a basis for identifying those who do and those who do not benefit from treatment as currently rendered. Comparisons are also being made with findings for outpatients in order to determine whether the predictive power of initial behavior patterns is similar for inpatients and outpatients. Data collection has been completed and the analyses are now under way.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02142-02 LDP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Behavioral Studies of Children with Juvenile Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	Blanche S. Jacobs	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Local Community Physicians in Private Practice

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.15	1.40	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this research is to identify attitudes, concerns and response patterns that children and their parents exhibit in relation to the child's having juvenile onset diabetes for a period of years. The main focus of the research is on identifying stabilized behavioral or adaptive patterns that have evolved as a psycho-social reaction to the disease and to ascertain the factors that have led to a particular style of adaptation. Particular attention is given to whether a child develops a passive-helpless orientation or attempts to master and/or excel in some area of endeavor as a life style in coping with a chronic illness. The research involves the study of children between 11 and 18 years of age who have had juvenile onset diabetes for three years or longer. A matched control group of healthy children is also seen. Data collection consists of interviews, questionnaires, and standardized tests administered to the children and their mothers and the procurement of life event data from school and medical records. This research is considered a prototype for how children in general cope with stress.

PHS-6040
(Rev. 10-76)

Project Description

Chronic and life threatening illness in childhood presents special challenges, conflicts, and stresses for the stricken child as well as for his/her family. The purpose of the research is to identify attitudes, concerns, and response patterns that children and their parents develop as a function of the child's being ill with juvenile onset diabetes for a period of years. A major focus of this research is on coping patterns and the acquisition and use of skills in dealing with a stressful life event. A variety of factors assumed to influence the child's stabilized modes of dealing with the illness are studied. Factors include severity of the initial episode, age of the child, family behavior patterns, support systems, health attitudes, personality attributes, and parental attitudes and behaviors. The research is considered a prototype for studying how children in general cope with stress. It is assumed that juvenile diabetes involves special and standard life stress situations that dramatize developmental processes and coping patterns that are characteristic of all children.

A central hypothesis is that the behavior of children who are handicapped by some chronic adversity will be polarized toward exhibiting extremes on the dimension of helplessness or heightened mastery behavior. Children who are living with a personal misfortune will tend to be bimodally distributed whereas healthy or unaffected children will be normally distributed on this dimension.

The research involves the study of 40 children between 11 and 18 years of age who have had juvenile onset diabetes for three or more years, by which time stabilized behavioral reactions to the illness should be manifest. A matched control group of children with no history of chronic illness or permanent handicaps also will be studied. These children and their mothers are seen at NIMH for two sessions lasting from 2-1/2 to 3 hours each. Each session will consist of an interview with the child, a separate interview with the mother, and the administration of a series of standardized psychological tests aimed at identifying personality characteristics, child rearing practices, goal setting behavior, and standards of competence. The first interview with the child and the mother focuses on the history, affective reaction, and adaptation to the illness and the second interview with the mother and child largely deals with the fostering and acquisition of coping styles, standards, and evaluating helplessness and mastery orientations in the child. For the control group, only the second interview and the psychological tests will be administered.

The battery of procedures administered in addition to the interview includes: (1) a level of aspiration task; (2) an anagram task; and (3) a categorical fluency task. Two Q-Sort procedures are also administered to both the child and mother in which they sort items which describe the mother's child rearing practices and psychological attributes of the child. The mothers also are administered the Jackson Personality Inventory.

This research is in progress and the initial cases have been studied. It is anticipated that about one year will be required to complete the data collection.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02143-02 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Psychosocial and Biomedical Interactions in Juvenile Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Beatrix A. Hamburg	Medical Officer, Research	LDP NIMH
OTHER:	Gale Inoff	Psychologist	LDP NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Irwin J. Kopin	Chief	LCS NIMH
	Phillip Gorden	Chief, Clinical & Cellular Biology Section	DB NIAMDD
	David Newsome	Chief, Retinal & Ocular Connective Tissue Diseases Section	CB NEI

COOPERATING UNITS (if any)
Laboratory of Clinical Science, NIMH
Diabetes Branch, NIAMDD
Clinical Branch, NEI

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.60
PROFESSIONAL: 1.80
OTHER: .80

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Stress significantly influences all aspects of health status and behavioral adaptation of diabetics. There are concomitant neuroendocrine effects as well as long-term outcomes reflecting level of adaptation to having a life-threatening chronic disease and engaging in the highly structured, stressful life style necessitated by the diabetes treatment regimen. This project compares metabolically-labile, insulin-dependent adolescent diabetics with non-labile diabetics and with non-diabetic, non-obese individuals under three specific conditions of stress -- interpersonal stress, emotional but non-interpersonal stress, and physical stress (exercise). It is hypothesized that there are significant individual differences in diabetic patients' patterns of biomedical response to stress which will systematically relate to whether the patients are labile or non-labile. Behavioral variables being studied include: coping patterns; locus of control; knowledge and skill regarding diabetes; denial, anxiety, and depression; self-esteem; family dynamics; and psychosocial functioning of patient and family.

Project Description

The study of juvenile diabetes is appropriate as a model for understanding the interactions of biomedical and psychosocial factors affecting medical and psychological well-being. In this program of studying psychosocial and biomedical interactions, the initial project involves comparisons of (1) metabolically labile, insulin-dependent adolescent diabetics, (2) non-labile diabetics, and (3) non-diabetic, non-obese individuals. Subjects are compared under three specific conditions of stress -- interpersonal stress, emotional but non-interpersonal stress, and physical stress (exercise). Stress significantly influences all aspects of health status and behavioral adaptation of diabetics. There are concomitant neuroendocrine effects as well as long-term outcomes reflecting the level of adaptation to having a life threatening chronic disease and engaging in the highly structured, stressful life style necessitated by the diabetes treatment regimen.

For many years, diabetes was viewed as a single hormone deficiency syndrome in which the disturbances of glucose metabolism could be reversed by exogenous insulin administration. Recent advances in diabetes research have established the multifactorial control of glucose regulation. The most prominent of the regulators that are known to increase blood sugar are also significantly responsive to stress. These include: growth hormone, glucagon, catecholamines, and cortisol. Recent studies of neuropeptides have shown that somatostatin can be a powerful inhibitor of both growth hormone and glucagon. The role of other neuropeptides in glucose metabolism is less clear. It has also been shown that physiologic increments of glucagon, epinephrine, and cortisol have synergistic actions. This work suggests that stress hyperglycemia cannot be ascribed to a single factor but is a consequence of the interactions of several regulators. It is hypothesized that there are significant individual differences in diabetic patients' patterns, of biomedical response to stress which systematically relate to whether the patients are labile or non-labile.

Some clinical approaches to the treatment of labile diabetic patients involve the assumption that the lability is due to interpersonal stress reactions stemming from specific intra-familial conflicts and particular disturbed patterns of family functioning. The present study examines this model by assessing biomedical responses to interpersonal stress during an experimental procedure involving the family. Behavioral observations and systematic psychosocial assessments also are made during this family interaction procedure. Additional characterization of family dynamics is obtained from interview data. Biomedical responses to emotional, noninterpersonal stress and to the stress of exercise also are examined. It is hypothesized that labile diabetics have aberrant biomedical patterns of response to stress but that these patterns of response may be triggered by emotional, non-interpersonal stresses as well as by those of an interpersonal nature. The pattern of biomedical response to exercise stress is examined for evidence of aberrant metabolic response as a possible characteristic of labile patients.

The procedure for assessing interpersonal stress involves a triadic version of the Color-Match Test. In this conflict resolution task, "challenge" colors are presented to the subject and parents to match by comparison with colors before each of them on individualized color boards. After independent judgments are announced, subjects must reach a consensus judgment on a single color to be chosen for each of the 30 trials. In one-half of the trials, non-compatible color stimuli are experienced by each participant. Thus, disagreement is built into the task.

There are two procedures for assessment of emotional but non-interpersonal stress. One involves a modified version of the Stroop Color-Word Interference Test. The second involves an ophthalmologic examination. For the Color-Word Interference Test, the subject names the colors in a set of color words printed in incongruous colors (e.g., the word "red" is printed in blue; the subject should respond "blue"). The test will be modified to include another simultaneous interference -- names of other incongruous colors which are presented aurally. Presentation of stimulus words is paced at a fast reading speed. The demanding nature of the task is frustrating and, thus, emotionally stressful.

The ophthalmologic examination is assumed to constitute a significant emotional stress for diabetics. Retinopathy is an early and common complication. Typically, 15 years after the onset of diabetes, about 60% of IDD patients have proliferative retinopathy. This is well known to diabetics. The examination is performed by an ophthalmologist of NEI and is limited to subjects who are already aware of the relation between diabetes and retinopathy.

The physical stress involves exercise using a cycle ergometer initially at an intensity of .5W/kg body weight and progressively increased until the subject achieves a heart rate of 120 beats/min. within 2-3 min.; total duration of exercise is 15 minutes.

Behavioral variables to be studied include: coping patterns; locus of control; degree of knowledge and skill regarding diabetes; denial, anxiety, and depression; self-esteem; family dynamics; and psychosocial functioning of patient and family. Assessments are being made while subjects are in-patients at the NIH Clinical Center for a duration of one week.

Publications:

Hamburg, B. A.: Developmental Issues in School-Age Pregnancy. In Bowers, J. Z., and Purcell, E. F. (Eds.): Aspects of Psychiatric Problems of Childhood and Adolescence. New York, Josiah Macy, Jr. Foundation, 1980.

Hamburg, B. A., and Hamburg, D. A.: Life Span Perspective on Adaptation and Health. In Kaplan, B., and Ibrahim, M. (Eds.): Family and Health: An Epidemiological Approach, Vol. II. University of North Carolina Press, Chapel Hill, North Carolina, 1980, in press.

Hamburg, B. A., Lipsett, L. F., Drash, A. L., and Inoff, G. E. (Eds.): Behavioral and Psychosocial Issues in Diabetes: Proceedings of the National Conference. U. S. Department of Health and Human Services, Public Health Service Publication No. 80-1993, Washington, D. C., U. S. Government Printing Office, 1980, in press.

Hamburg, B. A., Lipsett, L. F., Drash, A. L., and Inoff, G. E.: Behavioral and Psychosocial Issues in Diabetes: Summary Proceedings of the National Conference. Diabetes Care. March/April, Vol. 3(2), 1980, pp. 379-381.

Miller, B., Hamburg, B. A., and Kraemer, H. C.: Assertiveness Training for Adolescents: A Pilot Study. In Kaplan, B. (Ed.): Monograph Series on Family and Health, Vol. II. University of North Carolina Press, Chapel Hill, North Carolina, 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02144-01 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of Child Rearing

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
	Michael Chapman	Staff Fellow	LDP	NIMH
Other:	Leon Kuczynski	Foreign Visiting Fellow	LDP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .34	PROFESSIONAL: .30	OTHER: .4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The development of a research strategy by which parental rearing functions and modes of behaving in the parent role are kept intact, but by which experimental control over selected functions or events is also exercised is an objective of this research. A laboratory setting is used that simulates a home setting in basic respects and permits childrearing functions and daily routines to be carried out. This research paradigm is the basis of a series of investigations. In this project a major focus is on the conceptualization and measurment of rearing. Specific aspects of rearing (affective communications and control techniques) are investigated in Projects MH 02152 and MH 02155. Familes selected in terms of criteria of psychological disorders and of normality are investigated (MH 02156).

PHS-6040
(Rev. 10-76)

Project Description

In most theories, the environment in which a child is reared is considered a major contributor to the child's development. Measurement of the familial environment is difficult, however. Conceptualization of research dimensions and variables, accessibility of the phenomena to be studied, and techniques of assessment present enormous problems for research. The development of a research strategy by which parental rearing functions and modes of behaving in the parent role are kept intact, but by which experimental control over selected functions or events is also exercised is an objective of this research.

Implicit in developmental questions concerning rearing influences is the assumption that child behavior is shaped by the conditions provided by parents or their substitutes. (This does not exclude the idea of reciprocal influences of parent and child.) A longitudinal dimension or a rearing history of some duration is an essential in the study of environmental influences on child behavior.

A laboratory setting is used that simulates a home setting in basic respects and permits childrearing functions and daily routines to be carried out. Mother and child(ren) will spend half days in this setting; a number of half days are closely spaced in time, followed by an interval of weeks, followed by a closely spaced block of sessions in order to observe the development of specific responses or response patterns (their emergence, change, generalization, and so on). The spacing and the length of rearing measurements are in preliminary testing and will vary for different kinds of research.

The half-day sessions are programmed to include events and interactions that involve mother and child(ren) in physical care, stimulation, control, teaching, affective interaction, and solitary self-oriented pursuits. Assessment, includes behavioral, cognitive, and affective responding.

This research paradigm is the basis of a series of investigations. In this project a major focus is on the conceptualization and measurement of rearing. Specific aspects of rearing (affective communications, control techniques) are investigated in Projects Z01 MH 02152 and Z01 MH 02155. Families selected in terms of criteria of psychological disorders and of normality are investigated (Z01 MH 02156).

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02145-01 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Young Children's Responses to Expressions of Anger and Affection by Others in the Family

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Mark Cummings	Staff Fellow	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.80	.60	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The focus of this research is the impact on children of expressions of anger and affection by others in the home. Mothers trained as observers recorded the responses of 1-to-2 1/2-year-old children to expressions of anger and affection by others (usually family members). Children were always bystanders to these affective expressions. Anger and affectional incidents were responded to very differently. Affection observed between significant persons most often brought out expressions of pleasure or affectionate behaviors from the children. However, many of the affectionate responses had a jealous quality. Observed episodes of anger tended to elicit distress signs from the child, or no observed emotion. The probability of a distress response to anger episodes was heightened when angry interactions involved hitting. Repeated exposure to interparent anger increased the likelihood of an emotional reaction and also of more complex reactions by children. There were no age or sex differences in responses to anger or affection in others. The demonstrated sensitivity of young children to others' affects suggests the need to regard others' emotions as important influences in rearing environments.

PHS-6040
(Rev. 10-76)

Project Description

Others' emotions, even when not directed toward the child, may have an important impact on the child's well-being and development. Since there is little by way of accumulated research knowledge, a descriptive base of children's immediate responses to affect in others is a necessary starting point. This investigation examined children's reactions to others' (usually family members) expressions of anger and affection. Twenty-two children from white, middle-class families were studied for a 9-month period. Seven children were 10 months old at the onset of the study, 9 were 15 months old, and 6 were 20 months old.

Mothers trained as observers provided narrative accounts of behaviors during naturally occurring and simulated instances of anger and affection expressed in the child's presence. The adequacy of the mothers' training as observers is indicated by the high level of agreement between mothers' and researchers' reports of simulated anger incidents.

Anger and affectional incidents were responded to very differently. Affection observed between significant persons most often brought out expressions of pleasure or affectionate behaviors from the children; however, many responses from the children had a jealous quality. Observed episodes of anger, by contrast, tended to elicit signs of disturbance from the child, or no observed emotion at all. The probability of a response of distress from children was heightened when angry interactions involved hitting. Children who were repeatedly exposed to interparent anger more often showed complex and involved reactions. These children were the only ones ever to attempt to comfort or reconcile the angry parents. There were no age or sex differences in responses to anger or affection in others.

Thus, by one year of age, children are detecting from the background of other social interactions affective exchanges between others. They are also likely to evidence a clear emotional reaction to these interactions. The distinctly different responding to anger and affection demonstrates that young children are able to discriminate between others' expressions of emotions, even when emotions are not directed to the child. The demonstrated sensitivity of children to the emotions of others suggests the significance of these events as rearing variables.

This is a final report.

A manuscript of the report has been submitted for publication.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02146-01 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Etiology of Problem Aggression in Early Childhood

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Mark Cummings	Staff Fellow	LDP NIMH
OTHER:	Marian Radke-Yarrow	Chief	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.50	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The focus of this work is the early childhood etiology of problem aggression. Groups of problem aggressive and control children at several different ages are compared in terms of the following questions: (1) How do children behave in aggressive and nonaggressive interactions with family members and peers? (2) Are there distinctive ways in which others act towards aggressive and non-aggressive children? (3) What explanations do children provide for their own, and others', aggressive behavior? There are two primary sources of data. The first is mothers' observations, reported in narrative, of aggressive incidents involving the child during the daily course of events in the home. The second source of data is investigators' reports during home visits of the general interactions of children with family members and peers.

PHS-6040
(Rev. 10-76)

This research is designed to investigate the behavior of problem aggressive children in early childhood. Three specific issues are addressed: (1) How do problem aggressive children behave in aggressive and nonaggressive interactions with family members and peers? (2) Are there distinctive ways in which others act towards problem aggressive children? (3) What verbal explanations or reasons do problem aggressive children provide for their own, and others', aggressive behaviors? Several different ages are studied to allow a developmental examination of these issues.

Three-, six-, and nine-year-old problem aggressive and control children are selected on the basis of scores on the aggressive scale of Achenbach's Child Behavior Profile. Data are collected both by investigators and the children's mothers. Mothers are trained to provide detailed accounts of aggressive incidents involving the child during the daily course of events in the home. Investigators periodically visit the home to obtain data on the general interaction tendencies of the child and others in the home environment. Observations are also made by mothers and investigators of the child's interactions with peers. Finally, children are asked to respond to questions concerning their own and others' acts of aggression.

Data collection is under way.

Publications:

Cummings, E. M. Caregiver stability and day care. Dev. Psychol. 16: 31-37, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02147-01 LDP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Reasoning and Power Assertion in Parental Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Kuczynski	Visiting Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.40	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation is concerned with mothers' use of reasoning and power assertion in the course of controlling their children's behavior. Data on these disciplinary techniques are based on mothers' own trained observations of their reactions to children's misbehaviors in the home as well as observations of mothers and children in a laboratory setting. One focus is the study of the kinds of reasoning mothers use for children of different ages and in response to different misbehaviors. The determinants of mothers' choice of reasoning in response to different misbehaviors and in response to reoccurrences of the same misbehavior over time are also studied.

PHS-6040
(Rev. 10-76)

Project Description:

In the present study, the role of "reasoning" and "power assertion" in controlling children's behavior is investigated. A longstanding assumption in socialization theory is that reasoning (the provision of explanations for commands and prohibitions) promotes longterm changes in behavior and facilitates conscience development while "power assertion" (unexplained commands, punishments, rewards) promotes short term changes and hinders conscience development. This view of discipline may be inadequate since recent findings suggest that the effects of reasoning depend on the nature of the explanation given.

One aim of this study is to describe the kinds of reasoning mothers use with children. Reasoning as a technique has never been adequately conceptualized and has often been used as a blanket term to describe numerous verbalizations to children. No studies exist which investigate the forms reasoning takes at different ages and for different misbehaviors.

A second problem that is investigated in the relationship between reasoning and punishment in children's experience of discipline. Numerous studies have found that both reasoning and power assertion have important places in normal parental repertoires. However, little is known about how these techniques are used. When do parents primarily rely on use of force in controlling behavior? When do parents use reasoning alone or in combination with power assertion?

Finally, previous studies have assessed discipline only in response to single occurrences of misbehavior. What is not understood is how discipline is used over time with regard to the acquisition of specific aspects of self control. In the face of frequent occurrences of the same misbehavior are parents consistent in their use of a given disciplinary technique or so they change their disciplinary strategies?

Data for this project are obtained from children of several ages and their mothers. In the first phase of this study mothers' attempts to influence their children's behavior will be observed in the laboratory. In later phases, mothers will be trained to record their children's misbehaviors as well as their own responses to them in the home. Special attention will be paid to the provision of detailed descriptions of the reasoning used on these occasions and to the daily descriptions of mothers' reactions to selected chronic misbehaviors of their children.

Data collection for the first phase is under way. Data collection for the second phase will begin shortly.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02148-01 LDP

PERIOD COVERED October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Bilirubin as a Predictor of Dysfunction in Pre-term Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Sarah L. Friedman	Guest Worker	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.20	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children born premature are at risk for problems in physical, cognitive and affective development. One potential biochemical precursor of dysfunction is (blood) bilirubin. Bilirubin, which causes jaundice, is neurotoxic: At high levels (>20 mg. %) it causes mental retardation. Bilirubin levels in preterm infants are often elevated. It is hypothesized that a significant portion of the diverse, adverse sequelae of preterm births may result from the presence of this neurotoxin, bilirubin, which damages many regions of the brain shortly after birth. The purpose of this research is to determine if the amount of impairment in early affect expression (cries), neurological performance and visual function in preterm infants is associated with concentration of bilirubin in the blood.

PHS-6040
(Rev. 10-76)

Project Description:

Preterm infants (i.e., those infants born before 37 weeks' gestation) constitute a population that is at risk in several respects. Preterm, more than full term infants, experience (a) mortality and morbidity, (b) physical health problems, (c) impaired cognitive functioning, (d) visual problems, (e) learning and reading disabilities, (f) difficulties in regulation of affect, (g) child abuse and neglect, and (h) overprotection. While some of the correlates of preterm births have been identified and while many of the problems associated with early birth are known, direct causal connections between specific pre- and post-natal processes and specific biological and behavioral outcomes are difficult to infer. It is possible that a significant portion of the diverse, adverse sequelae of preterm births are the result of the neurotoxin bilirubin, which damages many regions of the brain shortly after birth. The purpose of this research is to determine if the amount of impairment of early affect expression (cries) and motor and sensory performance of preterm infants is associated with the concentration of bilirubin in the blood.

Behavioral data already have been obtained on 45 preterm infants in individual testing sessions at the Washington Hospital Center. Each child was tested around expected date of birth (38-42 weeks conceptional age). The measures include (1) visual attention -- measured in terms of orientation, attention level and response decrement to a repetitive visual stimulus, (2) neurological function -- assessed by the Parmalee neurological examination, and (3) affect expression -- measured in terms of (a) irritability, soothability and activity recorded during time samples of observations of infants' behaviors and (b) infant cries tape-recorded during the neurological examination. The biomedical and medical data are in hospital records and informed consents for this information are currently being obtained. Data analyses will begin when this step has been completed.

The study of preterm infant cries is of special interest because it may begin to provide a link between some of the biological and behavioral problems faced by significant numbers of preterm infants. The preterm cry has been described by some investigators as aversive and stressful. If this is true, then the children who show more stressed and disorganized affect may place significant additional stresses and requirements on their caretakers. Some preterm infants are said to be at risk for abuse and neglect: Their disorganized affect might be thought to elicit similar behavior in the caretaker. For other caretakers the cry of the preterm infant may function as a signal that special care and support are needed. Our understanding of the complex interactive processes that constitute socialization should be enhanced by knowledge of the origin of the affective stimulus properties that the preterm child brings to its environment.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02149-01 LDP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mothers' and Children's Reactions to Infant Cries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Sarah L. Friedman	Guest Worker	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .25	PROFESSIONAL: .20	OTHER: .05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The infant's cry is an important communication signal. It conveys information about the state of the young organism and evokes significant affect and behavior in others. The cries of pre-term and full-term infants are used as distress stimuli to study parents' and children's responses to different types of infant cries. Preterm cries have been described as especially aversive and potential elicitors of child abuse. Sixty-one mothers rated 12 cries on five 7-point scales, with negative polarity representing the urgent grating, sick, arousing and immature qualities of cries. Sixty-one children (age range, 4-11 years) made ratings of one preterm cry and one full term cry along dimensions of anger, fear, avoidance and empathy; also their emotion expressions and behavioral reactions to a crying infant were measured. Both mothers and children differentiated preterm from full term infant cries. For mothers, cries of moderate risk preterms were consistently rated more negatively than full term cries. Cries of some low risk infants were rated more negatively, but others more positively than cries of full terms.

Project Number: Z01 MH 02149-01 LDP

Project Description:

The infant's cry is universally recognized as an important communication signal. The cry simultaneously conveys multiple affective and cognitive messages to others in the young child's environment. Distress cries provide signals for routine caretaking, displays of affection, and often communicate to others the more serious needs encountered by the infant. As such, the cry can provide an adaptive function for young organisms, evoking attention from others and relief for the infant in distress. Infant cries also have aversive characteristics which are stressful to others and which can culminate in expressions of fear, anger or tension in relation to the child. Thus, qualities of the cry may target the child for less than optimal caretaking. Different cry characteristics of different infants are considered by some investigators to be one of the significant determinants of how others will respond to cry signals. The purpose of this research is to explore whether and how differences in cry characteristics influence the reactions and interactions of others with the infant. Children's and adults' behaviors are examined in response to the cries of full term and at risk (preterm) infants. Preterm cries have been described as more aversive and arousing than full term cries and as potential elicitors of aggression from caretakers.

In a laboratory study, sixty-one mothers rated 12 infant cries (4 full term, 4 preterm, moderate risk; and 4 preterm, low risk) on 5 seven-point scales, with negative polarity representing the urgent, grating, sick, arousing and immature quality of cries. The children of these women, ranging in age from 4 through 11 years, made ratings on a 3-point scale of one preterm cry and one full term cry along dimensions of anger, fear, aversiveness, and empathy. Earlier in the session, the emotional and behavioral reactions of these children were measured as they overheard a crying infant in an adjacent room. Half of the children heard one of two preterm infant cries and half of the children heard one of two full term infant cries. Children's responses to the cries were videotaped. To provide a natural context for the cries, all of the children, earlier in the session, had seen a mother with her infant. After hearing the cry, the child encountered the mother and infant again, and the child's behaviors toward the infant were measured.

Both mothers and children differentiated preterm from full term infant cries. Mothers consistently rated cries of moderate risk preterms more negatively than cries of full terms. Cries of some low risk infants were rated more negatively, but others more positively, than full term cries. At a descriptive level, a significant number of children characterize the cry of a preterm infant in non-human terms (e.g., "a sheep," "a donkey," "a ghost") or in terms descriptive of extreme perceived distress ("like it is being stabbed by a pen"). From these data, in conjunction with other of the data yet to be analyzed, we aim to begin to provide information about the different cries as they potentially may impact on the environment in which the child is reared.

One manuscript has been submitted for publication and another is being prepared for publication. The data on mother's responses to cries has been accepted for presentation at the APA meetings in Montreal, September, 1980.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02150-01 LDP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Effect of Transition on Children's Social Competence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Editha D. Nottelmann	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

Montgomery County Public Schools

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.30	PROFESSIONAL: 1.00	OTHER: .30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study is focused on transition from elementary school to middle school and to junior high school. The effect of discontinuity on children's social competence is investigated. Standard group instruments will be used for a multi-faceted assessment of children's social competence before and after transition, including (a) children's assessments of their own social competence, in relation to their perceptions of their cognitive and physical competence and feelings of general self-worth; (b) teacher ratings across the same domains of competence; (c) sociometric peer ratings; and (d) children's reflected peer and teacher ratings. These indices will be related to each other within and across three times of measurement. They will also be examined in relation to the children's views of their school environments. In addition, children's social competence will be considered in relation to data on physical maturation and performance in school.

PHS-6040
(Rev. 10-76)

Project Description:

This study is focused on the relationship between children's social competence and experience with discontinuity in their lives. Specifically, children are studied in transition from elementary school to middle school and to junior high school. Their social competence is examined across school settings, from their own perspective as well as from that of their teachers and peers, before and after transition.

The transition from primary to intermediate school has been selected for study, because it represents significant, naturally occurring change for a large number of children in our society. It involves change that is sufficiently far reaching to constitute a sharp discontinuity in children's lives. Many development and social problems of children appear at this time. Although children's social competence in one setting has been found to be predictive of their competence in similar settings, there is evidence that this predictability does not extend to new or different social environments. The intersection of the demands of new environments with the developmental demands and issues of later childhood and adolescence is investigated.

In this study, we are concerned with children's social competence; that is, with how they feel about themselves, with their ability to make friends and find peer support, and with their general social adjustment and psychological well being. Also, we are concerned with how their social competence is influenced by major change in their social networks and social environment. The research is designed to clarify how children's experience with school-peer discontinuities may change their self-perceptions and affect the way they are perceived by and interact with others. Measurements are made early in the period of transition and also later, after they have had an opportunity to adapt to their new environment.

This study represents a first step in the investigation of the effects of discontinuities in children's lives on their interpersonal relations in the three basic social systems in which they participate: the family system, the peer system, and the school system. The present study is limited to the intersection of the peer and school systems. It will provide the data base for a subsequent assessment of children's relations with their parents and peers outside of school, which, in turn, may be expected to influence children's social competence in school.

Data collection is scheduled to begin before the end of the 1979-1980 school year.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02151-01 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Social Cognition and Cognitive Capacity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology, NIMH

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .30	PROFESSIONAL: .20	OTHER: .10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relation of children's social cognition to other cognitive abilities is investigated. In particular, social perspective taking as assessed by the Chandler Bystander task is compared to backward digit span performance, a commonly used measure of working memory (Case, Pascual-Leone). From an analysis of the Chandler task, it is predicted that the ability to hold and coordinate three items in working memory is prerequisite for a nonegocentric response on this task. Data collection for an initial study is complete, data analysis is under way, and the preliminary results which are available support the hypothesis.

PHS-6040
(Rev. 10-76)

Project Description:

Children's social perspective taking is investigated in relation to other types of cognitive abilities. Cognitive developmentalists have generally held that perspective taking is a function of decentration and have supported their view with evidence that children begin to give nonegocentric responses on commonly used perspective taking tasks at about the same age as they begin to solve other types of tasks (e.g., conservation) thought to involve decentration. Others have emphasized the importance of the "cognitive difficulty" of particular perspective taking tasks and have demonstrated that, if tasks are simplified in certain respects, even young children seem capable of taking another's perspective. The problem is that there has been no common agreement as to what constitutes the "cognitive difficulty" of different tasks. The present project represents an effort to evaluate the "difficulty" of perspective taking tasks in terms of the demand placed by those tasks on subjects' working memory (Case, Chapman, Pascual-Leone). From an analysis of the Chandler Bystander perspective taking task, it is predicted that only children who are capable of coordinating three independent items in working memory should be able to give a nonegocentric response on the Chandler task.

In an initial study, the Chandler task together with the Peabody Picture Vocabulary Test and the forward and backward digit span tasks from the Wechsler Intelligence Scale for Children were given to a sample of 40 4-, 5-, 6-, and 7-year-olds, equally divided between boys and girls. Backward digit span is a commonly used measure of working memory (Case, Pascual-Leone), while the Peabody test provides a general measure of verbal IQ. Preliminary results strongly support the hypothesis: without exception, only children who were able to reverse three or more digits also gave nonegocentric responses on the Chandler perspective taking task. Verbal IQ was also correlated with perspective taking, although not as strongly as backward digit span. These results indicate that this approach to the study of perspective taking and of social cognition in general is worthy of further investigation. From detailed analyses of perspective taking tasks it should be possible to specify exactly what items (schemes) are involved in perspective taking performances.

Publications:

Chapman, M. Pascual-Leone's theory of constructive operators: An introduction. Human Development, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02152-01 LDP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Discipline and Parental Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Kuczynski	Visiting Fellow	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	Michael Chapman	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

—

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .60	PROFESSIONAL: .45	OTHER: .15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation is concerned with the measurement and conceptualization of parental control of children's behavior. This study is part of a series of investigations, the basic research paradigm for which is described in Annual Report MH 02144-01. Children's misbehaviors and mothers' discipline and control techniques are observed during a series of half-day sessions in a laboratory setting that simulates a home environment. Data on discipline will be based on naturally occurring controls placed on children in the laboratory setting as well as experimentally elicited disciplinary interactions. The range of control techniques used by mothers, the effects of these techniques on children's behavior and the role of children in influencing the kinds of discipline used by parents are investigated. Another focus is on the relationship between specific kinds of misbehaviors and the kinds of controls elicited by them. Do different kinds of misbehaviors elicit different characteristic patterns of control techniques in the same parents? Finally, the acquisition of specific aspects of self-control over time and parents' reactions to reoccurrences of the same misbehaviors will be documented.

Project Description:

This study is concerned with issues of parental discipline and control over children's behavior. Other aspects of child rearing are investigated in Projects 02144-01, 02147-01, and 02132-02 of this series. Central to the present investigation is the conceptualization and measurement of parental control. Theory and research on socialization has traditionally been guided by inadequate conceptualizations of parental control. Chief among these has been an excessive reliance on a unidirectional model of discipline in which parents' control techniques are considered only in terms of their influence on children's behavior. Recent studies, however, have indicated that children can modify parents' disciplinary strategies. A promising approach has been to regard the nature of children's misbehavior as an important determinant of parents' choice of discipline. An important hypothesis is that specific categories of misbehaviors may elicit characteristic forms of discipline from parents. One aim of the study is to investigate parents' choice of discipline in response to different categories of misbehavior.

A second characteristic of the socialization literature has been to describe parental control techniques in terms of a limited number of global categories of discipline such as "reasoning" or "punishment". Typically, these categories have been considered as unitary constructs as if all forms of reasoning have one, identical set of effects and all forms of punishment have another, identical set of effects. Observational research that has begun to examine the role of previously unexplored forms of control also suggests that parental discipline has been conceptualized too narrowly. A second focus of the present research will be to describe in terms of more discrete categories than previously the range of parental control techniques that children experience and to describe their influences on children's behavior.

Another purpose of this investigation is to study children's acquisition of specific aspects of self-control over time and to observe parents' reactions to reoccurrences of the same misbehaviors. Most of the available data on the effects of discipline are based on discrete sequences of disciplinary interactions. Usually, the effects of single administrations of control techniques on children's immediate compliance are studied. In the natural environment, however, children are likely to commit the same transgressions repeatedly over time, on each occasion eliciting different controls from their parents before the behavior finally comes under control. The present study will attempt to document in greater depth than previously the acquisition of specific aspects of self-control both in terms of children's behavior and parental discipline. Only a longitudinal analysis of discipline will permit researchers to go beyond the study of immediate effects of specific control techniques to a consideration of longterm influences and additive effects of discipline.

Data for this project will be gathered from observations of mothers and their children in a series of half-day sessions in a laboratory setting designed to simulate a home environment. Both naturally occurring controls placed on children by their mothers in this setting as well as experimentally elicited disciplinary interactions will serve as data.

Data collection is under way.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02153-01 LDP

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Maternal Recall of Child's Early Experience

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.:	Penelope K. Trickett	Senior Staff Fellow	LDP	NIMH
OTHER:	Marian R. Yarrow	Chief	LDP	NIMH
	Carolyn Z. Waxler	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL ~~MANYEARS~~: staff years .65	PROFESSIONAL: .55	OTHER: .10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, the relation between mother's recollections of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology. Although previous research has demonstrated that in general the correspondence between these two sources of data is low, there is still much to be learned about what determines when mother's recollections are more or less accurate. 1) Are certain kinds of information recollected more accurately than others? 2) How does the mother's current view of the child color her memories? The subjects are mothers of 26-month-old children who are part of a study of etiology of behavior problems. Mothers are currently observing and reporting child behavior and parental rearing behavior. They will also reconstruct their parallel observations of the child's behavior and their own behavior when the child was 18 months old (Z01 MH 00275).

Project Description:

Much of the knowledge in the field of child development on childrearing techniques has been derived from information obtained by interviewing mothers. Some of these interviews have been frankly retrospective, asking mothers to recall certain characteristics of her child or her child-rearing techniques when her child was at a younger age. Other interviews concentrate on the present but still rely on retrospection to the extent that they require the mother to use memories of previous experiences in order to make generalizations about her own or her child's typical behavior. In this study, the relation between mother's reconstructions of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology.

Previous research (Yarrow, Campbell, and Burton, 1970) has demonstrated that the correspondence between mothers' recollections and parallel information obtained at an earlier time is often low and that systematic biases in retrospection can occur. (For example, mothers tend to recall that their child was happier and healthier than they reported at the time). However, there is still much to be learned about the factors that determine when mothers' recollections and generalizations are apt to be more or less accurate. For example, are certain kinds of information recollected more accurately than others? How does the mother's current relationship to the child color her memories?

Mothers in the current research are part of a clinical study of etiological factors in the development of behavior problems of children. The children are now 26 to 28 months of age. The baseline data are (a) observational records by mothers who have been trained as observers and have been providing, on a longitudinal basis (10 to 24 months), rich data on their children's responses to emotions, transgressions, and prosocial behavior and on their own discipline techniques, (Z01 MH 00275), and (b) investigators' assessments of the mother-child relationship and of child behavior. The mother will reconstruct her child's and her own behavior when the child was 18 months old. The investigator will make ratings of current mother-child relationships. The several sets of data will be compared for correspondences and differences.

Data collection is underway.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02154-01 LDP

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparison of Individual Behavior Problems Before and After Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.	Jerome H. Blue	Staff Fellow	LDP NIMH
Other:	Thomas M. Achenbach	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL ~~MANYEARS:~~ staff years	PROFESSIONAL:	OTHER:
.48	.43	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this research is to compare data on individual behavior problems within a variety of mental health settings. The data were obtained in previous studies on several hundred children from residential and day clinics. Correlations will be performed between behavior items reported on the Child Behavior Checklist (CBCL) at intake and follow-up to assess stability and change in behavior over the course of treatment.

PHS-6040
(Rev. 10-76)

Project Description:

Previous analyses of data on the child behavior checklist (CBCL) have mainly focused on global measures of behavior. The present study is designed to make a more molecular analysis of individual behavior problems.

The design will entail an analysis of changes in each behavior problem reported at intake from a variety of mental health settings. Analyses will be performed to determine which problems remain the most stable and which ones are subject to greatest change. The sample will include data from several hundred children reported at intake and after treatment. The children were treated in residential and outpatient settings. Behavior ratings were obtained from parents, clinicians, observers and teachers. Statistical analyses are expected to show which problems are transitory and which are resistant to change. We expect to determine whether or not there is a difference between inpatient and outpatient care in terms of problems at intake which are found to be most enduring.

The findings are expected to aid in distinguishing between those problems reported at intake that are most resistant to change and those that may be less serious because they are transitory. These distinctions will help to guide treatment decisions as well as future research oriented toward the most critical behavior problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02155-01 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Affective Communication Patterns between Mother and Child

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Michael Chapman	Staff Fellow	LDP NIMH
	Leon Kuczynski	Visiting Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .75	PROFESSIONAL: .45	OTHER: .30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Affective communication patterns between parent and child are studied in two populations (families where there is psychopathological disturbance in the parent and also in non-deviant families). This study is part of a series of investigations, the basic research paradigm for which is described in Annual Report MH 02144-01. Disturbed and nondisturbed mothers will be observed with their children over an extended period of time in a home-like setting, under a number of naturally occurring rearing conditions conducive to the measurement of emotion expressions (e.g., pleasure, anger, distress) both in the mothers and in their children. Mothers will also be asked, on certain occasions, to simulate specified emotions (or lack of emotion) and systematically to vary an expression of mood (e.g., depression-dejection) on a short-term basis. The affective impact of these encounters on the developing child will be assessed. The mothers' reactions to and interpretation of others' emotions will also be measured.

PHS-6040
(Rev. 10-76)

Project Description:

Recent methodological advances have made it possible to obtain reliable and valid measures of emotion expressions in children and adults (e.g., expressions of anger, fear, distress, joy, surprise). Thus, research on the development and channeling of emotions is proceeding with renewed vigor. This project focuses on the antecedents of individual differences in the development of children's expressions of emotion, with special emphasis on the influences on children of variations in affective expressions of caretakers. Affective communication patterns between parent and child will be studied in families where there is psychopathological disturbance in the parent and also in normal families. Close-up examination of children with a parent with an affective disorder should help to aid in our understanding of differences in young children's emotional learning and development.

This study is part of a series of investigations, the basic research paradigm for which is described in Annual Report MH 02144-01. Disturbed and non-disturbed mothers will be observed with their children over an extended period of time in a home-like setting, under a number of naturally occurring rearing conditions conducive to the measurement of emotion expression (e.g., pleasure, anger, distress), both in the mothers and in their children. Mothers will also be asked, on certain occasions, to simulate specified emotions (or lack of emotion) and systematically to vary an expression of mood (e.g., depression-dejection) on a short-term basis. The affective impact of these encounters on the developing child will be assessed. The mother's reaction to and interpretation of others' emotions will also be measured: Mothers will be shown 35 photographs portraying a range of infant expressions of emotion and their interpretations will be obtained. Mothers' responses to different infant cry signals will also be measured.

Procedures are being developed and preliminary data collection has begun.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02156-01 LDP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of Child Rearing: Effects of Maternal Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Chapman	Staff Fellow	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Leon Kuczynski	Visiting Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.60	.25	.35

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Child rearing behaviors of parents with psychopathology (depression, schizophrenia) and the effects of these behaviors on children's development are studied. Children of psychopathological parents are known to be at risk for the development of psychopathology. This study will attempt to determine through what mechanisms parental pathology manifests itself in child rearing and affects children's behavior. It is predicted that affective communication and cognitive structuring are particularly important aspects of parent-child relations likely to be affected by parental pathology. Parents and children participate in half-day sessions in a home-like setting in the Laboratory of Developmental Psychology and their behavior in a series of standard situations meant to elicit typical child-rearing interactions is observed. This study is part of a series of investigations, the basic paradigm for which is described in Annual Report MH 02144-01.

Project Description:

The basic paradigm for the series of investigations of which this study is a part is described in Annual Report MH 02144-01. In the present study, a home-like environment is recreated in the laboratory and mothers are asked to participate in standard situations meant to recreate typical child-rearing interactions. For example, mothers will be asked to prepare a snack for the child, the child's reaction to the mother's absence and the presence of a stranger will be observed, mothers will be asked to teach children to perform age-appropriate tasks, and children's reactions to the experience of a physical examination will be observed, as well as the ways in which mothers control their children when they attempt to play with "forbidden" objects. The present study will focus on the analysis of mothers' and children's spontaneous behaviors in these standard situations.

Both verbal and nonverbal affective communication in mother-child interaction will be assessed. Facial expressions and bodily postures will be coded as the main channels of nonverbal affect communication. From previous experience with depressed parents, it is expected that depressed mothers will exhibit less affect communication with their children, less sensitivity to children's affect expressions, and less social stimulation overall. In contrast, clinical literature suggests that schizophrenic mothers may exhibit contradictory emotional messages, for example, expressing one emotion verbally and another nonverbally. Similarly, in regard to the cognitive structure (explanation of objects, persons, and events) provided by mothers for their children, it is expected that depressed mothers will simply provide less cognitive structure than normal mothers while schizophrenic mothers may provide irrelevant or inappropriate explanations by normal standards. Finally, it is expected that normal, depressed, and schizophrenic mothers will also differ in the amount and type of control of children's behavior shown in the laboratory setting. Depressed mothers may show less overall control than normal mothers, while schizophrenic mothers are expected to show a particular type of erratic, intrusive control.

While it is known that children of depressed and schizophrenic mothers are at substantial risk for the development of psychopathology, little is known about the precise behavioral mechanisms that are responsible. There are suggestions in the clinical literature that children themselves may become depressed as the result of the social-withdrawal characteristic of depressed mothers. On the other hand, the present researchers have found in previous research that children of depressed parents may develop surprising coping skills to obtain social stimulation from persons besides the mother. Likewise, little is known about the precise ways in which the child rearing behavior of schizophrenic mothers affects children's development. By observing mothers and children repeatedly, it should be possible in the present study to isolate some of the consequences of the child rearing patterns of mothers with these forms of psychopathology.

Pilot testing is complete and data gathering is under way.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02157-01 LDP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Developmental Evaluation of Infants on Chloride Deficient Diet

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
	Leon Kuczynski	Visiting Fellow	LDP NIMH
OTHER:	Van S. Hubbard	Clinical Associate	PMB NIAMDD

COOPERATING UNITS (if any)
Pediatric Metabolism Branch, NIAMDD

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .60	PROFESSIONAL: .40	OTHER: .20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In July 1979 it was discovered that a group of infants who were receiving Neo-Mull-Soy formula were not behaving and developing according to normative expectations and were exhibiting a "failure to thrive" type syndrome. Analysis of this infant formula revealed that it was deficient in chloride and that many of the infants fed this formula were suffering from metabolic alkalosis and chloride and potassium deficiences. Changes to a properly balanced formula improved their condition. There is some question, however, whether or not any permanent organic damage was sustained. These infants will be studied for a five year period to investigate the possibility of long-term effects from this dietary deficiency. Our research will focus on whether intellectual, behavioral, and child rearing practices were either directly and indirectly affected by the metabolic abnormalities produced from the use of this chloride deficient formula.

PHS-6040
(Rev. 10-76)

Project Description:

In July, 1979 it was discovered through an investigation conducted by the Center for Disease Control in Atlanta, Georgia, that a number of infants who were receiving Neo-Mull-Soy formula as their primary source of nourishment exhibited physical and behavioral abnormalities. These abnormalities consisted of weight loss, failure to grow, muscular weakness, delayed motor and speech development, chloride and potassium deficiences, and a condition known as metabolic alkalosis in which the pH levels of the blood are excessively alkaline. To date, about 120 infants who were fed either Neo-Mull-Soy or Cho-Free formulas have exhibited at least one episode of metabolic alkalosis. Analyses of these formulas have shown their chloride levels were about 1/5 of the amount recommended for infants by the American Academy of Pediatrics.

Once the formula was corrected there was remission of the metabolic abnormalities. The purpose of the research is to determine if there are any continuing complications or long-term sequalae associated with the chloride deficiency from the use of these infants formulas. This project is under the direction of Dr. Van Hubbard (NIH-NIAMDD) and will consist of extensive development studies of the physical and psychological growth of these infants as well as a complete evaluation of their biochemical, physiological, and neurological status, carried out at yearly intervals over the next five years. The Laboratory of Developmental Psychology is responsible for the psychological assessment of these children.

This study was recently initiated and some of the psychological procedures are still in the process of development. The children will be an average of 18 months of age at the time of the initial assessment. They will be administered an age appropriate measure of developmental or intellectual status at each annual assessment: the Bayley Scales of Infant Development to 24 months of age, the Stanford-Binet from 24-48 months, and the Wechsler Preschool and Primary Scale of Intelligence from 4 to 6-1/2 years of age. Assessment of parental and sibling IQ's will be obtained in addition to IQ scores on a matched peer group in order to have a basis for determining if the children who were on the chloride deficient diet exhibit any loss in intellectual functioning. The longitudinal design also will permit study of developmental changes and progressive decrements in IQ level over time.

Measurements of children's temperament as well as their compliance and responsivity to maternal directives are also included in this project. Recent studies have shown that mothers' use of control techniques are influenced by children's own behaviors. It is suspected that both mothers' controlling behaviors and children's compliance could be affected by problems of neurological development. Data are obtained from observations of mothers and children in a directed play situation. Mothers are asked to take an active role in controlling their children's behavior by directing them to attend to and engage in a predetermined variety of activities. The control and compliance data of this project will contribute to related research programs of the laboratory which are described in Projects MH 02152-01 and MH 02147-01. At older ages temperament and behavioral problems will be studied in greater detail to determine if this early chloride deficiency directly or indirectly influenced and disrupted these modes of functioning.

The mothers are interviewed about their perception of the developmental status of the infants, their anxiety, their fears, changes in their behavior and expectations concerning the child, the effects of this experience on family planning, and their attitudes toward and trust in institutions and other aspects of society.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02158-01 LDP

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Impact of the Rearing Environment on the Social/Emotional Development of the Abused Child.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.:	Penelope K. Trickett	Senior Staff Fellow	LDP	NIMH
	Elizabeth J. Susman	Senior Staff Fellow	LDP	NIMH
Other:	Ira S. Lourie	Assistant Director	DMHSP	NIMH

COOPERATING UNITS (if any)

Agencies and Institutions in the Washington Metropolitan area serving abusing families

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL ~~MANYEARS~~: staff years .85	PROFESSIONAL: .70	OTHER: .15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study focuses on the social and emotional development of physically abused children and the relationship between this development and the rearing environment of the child. While clinical evidence shows that abused children are at risk for a wide range of social and emotional problems, few controlled empirical studies exist and there is no research which relates aspects of the enduring child-rearing environment of the abused child's home to the child's development. This study will use a multi-method approach to obtain information about varied aspects of parental beliefs and practices involving discipline and other child control techniques. These methods include home observations, parent reports about daily interaction with their child, measures of parental attitudes towards different discipline techniques, and level of parental frustration tolerance. The social and emotional development of the child will be assessed in relation to affective coping, self esteem, interpersonal problem solving, and peer relations.

Project Description:

This study focuses on the social and emotional development of physically abused children and the relationship between this development and the rearing environment of the child. While there is wide agreement that the childhood victims of physical abuse are at risk for social or emotional maladjustment, few controlled empirical studies bearing on this issue exist. Psychiatric case studies and the experience of practitioners suggest that children who have suffered from physical abuse in their homes are likely to show diffuse symptoms of maladjustment, become abusers themselves as adults, or become runaways or have other troubles with the law during adolescence.

Research is needed to clarify how variable are the problems in social and emotional development of abused children and, especially, to investigate this over a wider age range than has been studied to date. It is also important to study the social and emotional development of abused children in the context of the rearing environment of their family because such development is certainly affected not just by the sporadic episodes of physical abuse, per se, but by the more enduring child-rearing environment of the child's home.

The present study will investigate in detail the child rearing environment, specifically those processes which may lead to abusive incidents and relate those to the social and emotional development of the child. There is evidence from several sources that frequently the immediate antecedents of parental abuse of a child involve parental discipline attempts or other efforts at control of the child. It is unclear, however, what the exact nature of this relationship is. Two competing hypotheses (which have been suggested as perhaps distinguishing different types of abusive parents) are (1) that abusive parents may believe that harsh physical punishment is a necessary technique if one is to rear a child adequately or (2) that abusive parents, while not valuing physical punishment any more than other parents, tend toward out-of-control anger episodes which result from child misbehavior.

The present study will use a multi-method approach to obtain information about varied aspects of parental beliefs and practices involving discipline and other child control techniques. Detailed reports will be obtained from the parents about their daily interaction with their child. These will provide information about the types and frequencies of child behaviors that result in discipline by the parent and the types of discipline techniques frequently used by the parent. Observations of the family in the home will also be made. Other measures will provide information about parental attitudes towards different discipline techniques and particularly corporal punishment and about the level of parental frustration tolerance when in interaction with the child. Information will also be obtained about the overall quality of the parent-child relationship and the family environment.

The social and emotional development of the child will be assessed in terms of affective coping, self-esteem, interpersonal problem solving, and peer relationships.

Subjects will be physically abused children ranging in age from 3 to 10 years of age and their parents. Recruitment of subjects from several local agencies serving this population is underway and data collection will begin soon.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00478-24 LN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in object perception and memory

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
OTHER:	L. Ungerleider	NEI Staff Fellow	LN NIMH
	B. Turner	Professor	Howard U.
	E. Murray	NIMH Extramural Fellow	LN NIMH
	J. Aggleton	NIMH Visiting Fellow	LN NIMH
	D. Friedman	NIMH Staff Fellow	LN NIMH

COOPERATING UNITS (if any)

Howard University

LAB/BRANCH
Laboratory of Neuropsychology

SECTION
Section on Cerebral Mechanisms

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.1	2.0	3.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate cortex is the souce of two major, corticocortical, multisynaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnecting the striate, prestriate, posterior parietal, and dorsal prefrontal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, inferior temporal and ventral limbic areas, and is critical instead for object vision. Our research concerns both pathways, but the accompanying report focuses on the second, or ventral one. Our studies suggest that each of the extrastriate links in this ventral pathway adds a different capability to object vision, making possible, in turn, highly efficient object perception, object memory, and object-affect associations. The sequential dependency of these behavioral functions is paralleled by the sequential dependency of the successive neural structures in the chain. The somatosensory cortex may also project through a ventrally directed pathway to temporal limbic areas, and parts of this system may perform functions in tactual perception, memory and affect analogous to those described above for the visual system.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The objective of the seven sub-projects described here is to define the role in object perception and memory of neural structures outside the primary sensory projection areas, using behavioral and anatomical analyses. (1) Monkeys were prepared with various lesions or injections of the lateral and medial striate cortex (area OC) and the brains studied with the Fink-Heimer silver impregnation and autoradiographic methods for determining cortical projections. Results have revealed three topographically organized representations of the visual field within the prestriate cortex: area OB, the posterior bank and depths of the upper superior temporal sulcus, and the depths of the posterior intraparietal sulcus. Analogous studies are now being carried out on the projections of area OB. (2) Monkeys are being prepared with as complete ablations as possible of the striate-projection zone defined in (1) prior to training them on visual pattern discriminations. The results will provide a rigorous test of the hypothesis that the prestriate cortex serves as the link between the striate cortex and the inferior temporal areas. Concurrently, to determine whether even small prestriate lesions might "disconnect" inferior temporal cortex from corresponding parts of the visual field, monkeys with such lesions will be tested for discrimination ability in different fields with the aid of a fixation-monitoring device newly developed for us by the Resource Services Branch. (3) Previous ablation studies had demonstrated that stimulus equivalence between the visual half-fields depends on the convergence of their projections onto single inferior temporal neurons. Follow-up studies using the fixation-monitoring device will test whether inferior temporal neurons account for stimulus equivalence across any retinal translation, and also whether, as suspected, it is the neurons in the posterior part of inferior temporal cortex that are mainly responsible for this stimulus equivalence mechanism. (4) Monkeys with various cerebral lesions were compared on tests designed to measure one-trial visual memory for objects. Only removals within the anterior part of the inferior temporal cortex (area TE) impaired this object recognition ability, suggesting that this is the locus in which the visual trace left by previous viewing is preferentially established. By contrast, on tests designed to measure one-trial memory of object-reward associations, equivalent impairments were obtained after area TE lesions and after amygdalectomy. The results thus point to object recognition and object-reward association as consecutive processes that depend in part on the sequential participation of IT cortex and the amygdala. New studies are now underway in an attempt to obtain more severe impairments in these two processes by increasing the size of the lesions in accord with information gathered in the 2-deoxyglucose experiments (see Project on Functional Mapping of Sensory Systems), showing the full extent of visually related areas. (5) In the process of investigating the role of temporal-lobe structures in memory we have chanced on a result that is particularly exciting because it may help to resolve a long-standing puzzle concerning the neuropathology underlying the syndrome of global anmesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. What we have found in our studies, both for visual recognition memory and for object-reward association memory, is that if the hippocampus is removed in combination with the amygdala then a profound memory loss ensues, one that is even greater than that produced by lesions of inferior temporal cortex. Furthermore, the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems. The discovery not only opens up a new possibility for resolving the discrepancy between clinical and animal findings, it also provides a new insight into the neural mechanisms of memory. Additional studies have been started in an attempt to (a) identify differences in the contributions of the amygdala and hippocampus to memory, (b) explore the interactions of these two limbic structures with the diencephalon, both anatomically and behaviorally, and (c) examine the nature and substrate of the memory ability that is preserved after destruction of the limbic system. (6) The discoveries in (4) and (5) were antedated and guided by anatomical findings indicating that inferior temporal cortex projects both directly to the amygdala and indirectly to the hippocampus via the entorhinal area. To extend and refine our knowledge of these anatomical circuits, we investigated with both axonal degeneration and transport methods the inputs to the limbic structures of the temporal lobe. Results thus far indicate that no visual area caudal to ares TE projects to the amygdala, but that all sections of area TE do, and that they do so within a circumscribed part of the basolateral nuclei. In addition, other sensory systems (i.e., olfactory, gustatory, somatosensory, and auditory) project to other parts of the amygdaloid complex in such a manner as to suggest that each sensory system has a dominant influence in a given part. These findings will help guide further behavioral studies into the possible mnemonic functions of the limbic system in all sensory modalities. (7) Monkeys are being trained on a tactual object recognition problem, to test the possibility that the amygdala and hippocampus participate in memory processes involving sensory systems other than the visual system, as the results of the anatomical studies outlined in (6) suggest. In addition, cortical localization studies are being performed in the somesthetic modality with the goal of identifying sequential processing systems in touch and kinesthesis analogous to those that have been identified in vision.

Scientific Significance to Mental Health Research: Vision is perhaps the best understood of the sensory modalities in relation to the functioning of the nervous system. Yet a major problem remains unsolved in vision as it does in all sensory modalities: Delineation of the events that intervene between stimulus reception at the cortex and the observed response. The demonstration that damage to the inferior convexity of the temporal lobes in monkeys produces impairment in visually-mediated behavior opened up the hitherto inaccessible area of the intracerebral processes in vision, i.e., neural activity related to vision but beyond the level of the striate cortex. Unravelling these mechanisms in vision and other modalities is aiding greatly in the solution of a general problem for psychology, viz., accounting for the intervening neural processes (thought and its breakdown) in normal and abnormal behavior.

Publications:

Mishkin, M. Inferior temporal cortex and stimulus equivalence across the retina. Seitai-No-Kagaku, 30: 436-450, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00480-16 LN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Development and Plasticity of Primate Frontal Lobe (Terminated)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Goldman	Chief, Section on Developmental Neurobiology	LN	NIMH
Other:	R. M. Brown	Senior Staff Fellow		NIDA
	L. J. Porrino	Staff Fellow	LN	NIMH
	N. Bugbee	NIMH Extramural Fellow	LN	NIMH
	S. Kojima	Visiting Fellow	LN	NIMH

COOPERATING UNITS (if any)

National Institute of Drug Abuse, ADAMHA,DHHS

LAB/BRANCH
Laboratory of Neuropsychology

SECTION
Section on Developmental Neurobiology

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Principal Investigator of this project, Dr. Patricia S. Goldman, left the NIMH-IR Program on September 30, 1979. Dr. Goldman is continuing this line of research in her position as Professor of Neuroscience in the Section of Neuroanatomy at Yale University School of Medicine.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02031-04 LN

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in spatial perception and memory

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
OTHER:	L. Ungerleider	NEI Staff Fellow	LN NIMH
	R. Nakamura	Staff Fellow	LPP NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

Section on Cerebral Mechanisms

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.9	PROFESSIONAL: 1.3	OTHER: 3.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate and prestriate cortex project not only ventrally, to the inferior temporal area, but also dorsally, to the posterior parietal and dorsal prefrontal areas. This dorsal projection pathway appears to constitute the visual contribution to a system for spatial perception. Many modalities undoubtedly contribute to this spatial system, which can be divided into a posterior (parietal) portion serving allocentric localization, and an anterior (prefrontal) portion serving autocentric localization. The entire system is probably critical for the spatial guidance of movement, and in keeping with this its major cortical outflow appears to be to the motor system. In addition, however, this spatial system appears to provide an essential tonic influence on the diffuse activating system, for its removal has been found to cause a profound sensory neglect.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The first two sub-projects described below deal with the parietal and prefrontal portions of the spatial system, respectively.

(1) Animals trained on a visual landmark test, a measure of allocentric localization ability, were prepared with posterior parietal removals in one hemisphere and complete striate removals in the other, and these were then followed by a callosal transection. Results indicate that the crossed lesions alone produced severe impairment and that, once the animal recovers, the addition of a callosal transection had only a mild effect. Thus, the posterior parietal cortex, like the inferior temporal, appears to depend heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive a heavy visual input via the corpus callosum. In a second experiment, monkeys received bilateral lesions of the striate areas representing either central vision (lateral striate) or peripheral vision (medial striate). They were tested both on a pattern discrimination task, to assess residual inferior temporal function, and on the landmark task, to assess residual posterior parietal function. On the former task, only the lateral striate lesion produced a deficit, whereas on the latter task, the two lesions produced equally severe deficits. Apparently, inputs from central vision are especially important for the pattern recognition functions of inferior temporal cortex, but inputs from central and peripheral vision are equally important for the visuospatial functions of posterior parietal cortex. Thus, although interactions with striate cortex are critical for the parietal just as for the temporal areas, the striate inputs to these regions appear to be organized differently, presumably reflecting differences in the cortical processing required for spatial vs object vision.

(2) A crossed-lesion experiment like that described above has shown that the dorsal prefrontal cortex also depends on striate input for its visual function, in this case autocentric localization as measured by the spatial delayed response test. Evidence that the function is selectively spatial comes from an experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests, including delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the principal sulcus relearned quickly, in sharp contrast to their permanent failure to relearn spatial memory tasks. The dimensions of the spatial deficit were examined in another experiment which compared the effects of selective prefrontal lesions on up-down delayed alternation as opposed to the customary left-right delayed alternation. In every case, lesions known to impair performance on left-right tasks (e.g., principalis removals, or inferior frontal ablations) had just as severe an effect on the performance of the up-down task, while removals known to have little efect on left-right alternation (e.g., periarcuate lesions) also had little effect on the up-down version. Ap-

parently, the spatial impairment after principalis lesions is not limited to left-right confusion, but encompasses the vertical dimension as well. Further support for the conception of a spatial impairment after principalis lesions was provided by an experiment on time discrimination. In this task animals were required to respond to one position after a short delay and to the other position after a longer delay, the two delays being presented in random sequence. Animals with principalis lesions were unaffected on this task, seriously undermining the view advanced by others that their delayed reaction impairment reflects a difficulty in temporally structuring, or "parsing", the stream of sensory stimulation to which they are exposed. The results suggest instead that the lesion produces impairment only when the spatial choice that the animals are required to make depends upon the memory of a spatial cue rather than the perception of a temporal cue.

(3) In another project (Neural mechanisms in object perception and memory) evidence was reviewed showing that visual discrimination habits depend on both the cortical visual system and ventral limbic structures. But are these two systems acting together a sufficient substrate for such habits, or does the rest of the cortex, including that in the dorsal spatial system, also play a significant role? To examine this question we restricted vision to a single hemisphere (by visually deafferenting and disconnecting the other hemisphere) and then removed all the cortex from the seeing hemisphere except for the visual and limbic systems. Unexpectedly, the operated animals appeared on awakening to be completely blind, and this total blindness lasted for one to several months, abating, if at all, only very slowly and only in part. Yet, the geniculostriate pathway in these animals seemed to be intact as judged both by visual evoked potential studies and from the subsequent histological examination of the brains.

To determine whether the blindness was the result of a disconnection of the visual system in one hemisphere from the motor system in the other, animals were prepared as before but with the forebrain commissures left intact. Yet these animals too were blind, indicating that the blindness is not a disconnection effect and suggesting further that during blindness the visual signal is not processed beyond striate cortex, for otherwise the signal should have been transmitted across the preserved commissural channels (which start at the striate-prestriate border) to the motor system. The analysis implies that the territory ablated normally provides some activating input, direct or indirect, that is necessary for cortical visual processing.

In a further study, the original lesion was subdivided in an effort to localize the area supplying the activating input. It was found that a sensorimotor lesion (Brodmann's areas 1-6) failed to produce blindness in any of three monkeys. The complementary lesion, however, consisting of dorsal prefrontal, inferior parietal, insula and superior temporal areas, did produce blindness in three monkeys for about a month. These localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a profound form of visual neglect, since parts of the lesion that produced blindness are known to produce neglect. Accordingly, the basis of the neglect too could be impaired processing within the cortical visual system due to loss of activating input, though a less complete loss than that producing neglect. It appears from these results that the dorsal "spatial" system normally provides an activating input (relayed perhaps through the activating system) that is necessary for the visual system and perhaps other sensory systems to function properly, and this is in addition to its contribution to spatial mechanisms. For when the animals eventually do recover their use of vision, they show permanent impairment in the ability to guide their body and limbs to visual targets; that is, they then show the expected spatial disorientation.

We have found, finally, that by extension of the lesion into the limbic system animals can be rendered chronically blind. Such preparations are now being used to test the loss-of-activation hypothesis directly by the method of unit recording in the intact visual system. Thus far, striate cortex was studied in five monkeys prepared as above to produce chronic blindness, except that the optic tract was left intact in two of the animals to provide control hemispheres. The recordings were made while the monkeys were anesthetized with nitrous oxide and immobilized with pancuronium bromide. Implanted wells and head holders were used to minimize possible sources of pressure or pain.

We isolated 244 units from the hemispheres made 'blind' by the ablation, and 83 units from the 'seeing' intact hemispheres. Of the units from the blind hemispheres, 53% were moderately or highly responsive visually as contrasted with 95% of those from the seeing hemispheres. The loss of visual responsiveness was related to a consistent histological finding of inadvertent damage to optic radiations representing primarily the lower visual field, in that there was a strong positive correlation between a cell's visual responsiveness and the vertical position of its receptive field. For example, at more than 5° below the horizontal meridian, only 12% of the units were moderately or highly responsive, whereas at more than 5° above the horizontal meridian, 71% of the units were moderately or highly responsive.

There was no difference between the blind and seeing hemispheres either in the proportion of responsive cells with oriented receptive fields (69% and 68%, respectively) or in the proportion of oriented cells with complex-type receptive fields (71% and 63%, respectively).

We conclude that 1) although many units respond poorly or not at all in the striate cortex of the blind hemispheres, there appear to be enough moderately or highly responsive units to support vision, particularly in the upper fields; 2) moreover, visual information is not only reaching striate cortex but is being processed there as indicated by the normal proportions of cells with oriented and with complex-type receptive fields; 3) the loss of visual processing or disconnection of the efferent visual pathway responsible for the blindness must be occurring at some point beyond striate cortex.

In addition to the unit-recording studies, we are currently pursuing the anatomical circuitry underlying the proposed activation. Our initial results suggest that the pulvinar nucleus in the thalamus may play an important role. We had previously found that striate projections to the prestriate cortical area OB are visuotopically organized, and we had prior evidence of a similar topographic arrangement of pulvinar projections to area OB. In subsequent studies, we found that there is also a precise visuotopic organization of striate projections to the pulvinar, indicating the existence of two sources of striate input to area OB that are in perfect register: one, direct, i.e., corticocortical; and the other, indirect, via the pulvinar. This parallel system of inputs to area OB thus provides a possible mechanism by which activating signals (e.g., from dorsal cortical areas to the pulvinar via midbrain structures) acquire visual field specification, that is, a mechanism which tells the organism where in the field to attend. Although this hypothetical circuit may apply for all sensory systems, for the visual modality our data suggest that only the inferior and lateral nuclei of the pulvinar are involved. This conclusion was inferred from the anatomical material from two series of rhesus monkeys in which striate projections to the pulvinar were mapped. Future anatomical studies will investigate the sources of midbrain input to PI and PL, as well as the organization of afferents to the pulvinar from other sensory modalities.

Significance to mental health:

Spatial orientation in animals is an extremely well developed, higher order, perceptual mode. It is "higher order" in the sense that it depends not on a single sensory modality but on the coordinated functioning of several of them. Our evidence points to a dual cortical localization for this suprasensory modality in the posterior parietal and dorsal prefrontal areas. Analysis of this complex, multimodal, cognitive system in monkeys may help us to understand the organization of yet another complex, multimodal, cognitive system in man, namely, the one for language. The formal similarities between the language system (for which man's left hemisphere is specialized) and the spatial system (for which man's right hemisphere is specialized) give added support to the validity of this approach.

Publications:

Ungerleider, L. G. and Mishkin, M. The striate projection zone in the superior temporal sulcus of Macaca mulatta: Location and topographic organization. J. Comp. Neurol. 188: 347-366, 1979.

Nakamura, R. N. and Mishkin, M. Blindness in monkeys following nonvisual cortical lesions. Brain Research 188: 572-577, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02032-04 LN

PERIOD COVERED

November 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neural Coding of Visual Stimuli

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. J. Richmond	Medical Officer	LN NIMH
OTHER:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
	R. Wurtz	Chief, Laboratory of Sensorimotor Research	LSR NEI

COOPERATING UNITS (if any)

Laboratory of Sensorimotor Research, NEI

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

Section on Cerebral Mechanisms

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.5	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous work in this laboratory and others has shown that the inferior temporal cortex is important for the visual processing underlying pattern recognition. Also studies have shown that single neurons in this area of cortex are responsive to visual stimuli. The studies which are underway are an attempt to determine what parameters or features of the stimulus are important in the modulation of the activity of the cells. We have chosen to do the analysis using spatial frequency gratings as the probe stimuli because all visual stimuli can be broken down into sine wave gratings, and thus the responses to sine wave gratings can potentially be used to reconstruct the response of single cells to more complex stimuli. The responses are compared to those obtained with stimuli which have been used previously such as bars, slits, and spots, as well as more complex patterns. We hope that by investigating single cell responses in this orderly manner we will be in a position to predict the stimulus parameters which are processed in this region of cortex, and as a result construct fruitful hypotheses regarding the role of inferior temporal cortex in stimulus coding.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives:

The inferior temporal cortex in the primate has been suggested as the site of visual pattern recognition. Experiments carried out in this laboratory indicate that tasks involving discrimination and recognition of patterns can only be carried out while these inferior temporal regions are intact. In the past, several single unit recording experiments have been performed here and in other laboratories to determine whether the inferior temporal region contains single neurons which show a stimulus locked response when visual stimuli are presented in the visual fields. These previous studies, which have been carried out largely in paralyzed and lightly anesthetized monkeys, have shown that the cells do respond to visual stimuli, and that they have large receptive fields which include the foveal or central region of vision. A problem which has troubled us, however, has been the difficulty in determining the important stimulus characteristics which cause the response of the cells. Also, when "conventional" neurophysiological stimuli have been used, responses have sometimes been difficult to obtain, and the optimum response to a visual stimulus is often found by chance only after prolonged searching through large arrays of objects. There has been no easy rule or set of rules which will allow the investigator to systematically vary stimulus characteristics in such a way as to alter the response of the cell predictably.

Over the past decade, important experiments on vision have been carried out in human subjects using the techniques of psychophysics. The experiments show that in some ways the visual system behaves as if it were preforming spatial filtering of patterns through "channels", where a channel is defined as a filter followed by a detector. A filter is an entity which responds to a particular characteristic of an input (stimulus) and yields a value reflecting the amount of that characteristic in the input. Of particular interest are the experiments which seem to show that the visual system acts as if it has a small number of channels sensitive to the spatial frequencies present in a pattern. One attraction of these results is the possibility they suggest of representing a pattern of visual stimulation as a ratio of spatial frequencies. Such a scheme would solve a potentially thorny problem posed by normal visual performance - namely the ability to recognize the same pattern at various distances, despite the change of image size on the retina. If the visual system uses ratios of responses in several different spatial frequency channels, it would then be rid of the size and distance interaction problem. Because of the large receptive fields and the role of the inferior temporal cortex in pattern recognition, the inferior temporal cortex is a prime candidate to serve as the substrate of the postulated spatial frequency channels. By using a set of basic spatial frequency stimuli (called sine wave gratings) we would hope to gain a better understanding of the neural mechanisms underlying some of the higher functions of the visual system.

The use of sine wave gratings has an additional advantage. Mathematically and optically the set of all sine wave gratings which are resolvable by the eye can be reconstructed to make up any visual stimulus. The methods for doing that are well worked out. Thus, even if the hypothesis that the visual system acts as a set of spatial frequency filters is incorrect, we may nevertheless be able to reconstruct the relevant stimulus features which will cause strong responses from the cells by collecting the data from the experiments in an appropriate form. Further, there is a possibility that such an approach will lead to an underlying rule or set of rules governing the response of the cells in this region of the cortex.

Methods Employed:

Two parts of the investigation have been accomplished so far. The first part has been to develop a device in order to present stimuli of the appropriate type and to connect it to our neurophysiological recording apparatus. The device is a large screen oscilloscope which is interfaced to a microcomputer and a special piece of custom hardware developed here at the NIH in conjunction with the Resource Services Branch of NIMH. With this visual stimulator we are able to present both conventional and spatial frequency stimuli with the speed and flexibility needed to perform the experiments we have undertaken.

The second part has been some initial single unit recording in the region of the inferior temporal cortex. We are using awake, trained monkeys in our experiments so that we will be in a position to observe any modulation of response by the cells which depend on the animal's attention, task, or motor response. This part has been carried out in two monkeys by means of the standard recording methods used in this laboratory.

Major Findings:

In the initial experiments carried out on 51 inferior temporal neurons we have been surprised to note that the receptive fields of the 36 visually responsive cells have been very small, much more like the receptive fields of cells in the primary visual cortex (striate cortex) than of cells previously described in the inferior temporal area. The excitatory parts of the receptive fields are very narrow, and the orientation of the stimuli is critical. If the orientation varies by as little as 15 degrees from the optimal orientation, the response to the stimulus may disappear entirely.

The length of an oriented slit stimulus is also often critical, with the strength of the response increasing as the slit is made longer up to a certain point, following which a further lengthening leads to a dramatic decline in the response of the cell to the stimulus (a stopped-end response). Many of the cells which respond to slit stimuli also show a good response to grating stimuli of a relatively low spatial frequency, e.g. 3-4 cycles/degree. Some of the visually responsive cells do not seem to respond with a marked increase in sustained firing rate to the grating stimuli. Often the cells which respond to either type of stimulus respond with a transient or "on" response, and then show an average firing rate somewhat above the initial background or pre-stimulus firing rate.

In summary, the cells which we have studied in the initial part of this experiment show a good response to conventional stimuli (in this case bars of light), the response is highly orientation specific, and many of the cells have a "stopped-end" type of response. The use of sine wave grating stimuli also elicits a good response.

Significance to mental health:

Solving the problem of how the brain processes sensory information has been one major goal of psychologists and neurophysiologists. These experiments are an attempt to move closer to an understanding of such processing in the visual system of the primate. Since the inferior temporal region is involved in higher order visual processing, we would expect that an understanding would not only further our knowledge of how this cortex codes and stores visual stimuli, but it should also give insight into perceptual and memory problems seen clinically as a result of acquired or congenital defects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02033-03 LN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Functional Mapping of Sensory Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN NIMH
OTHER:	K. Macko	NIMH Extramural Fellow	LN NIMH
	C. Kennedy	Guest Worker	LCM NIMH
	E. Murray	NIMH Extramural Fellow	LN NIMH

COOPERATING UNITS (if any)

Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH
Laboratory of Neuropsychology

SECTION
Section on Cerebral Mechanisms

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.7	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Converging evidence from studies of lesion effects, anatomy, and single units indicates that, in the primate, the system for processing information about visual objects (i.e. object vision) extends beyond the striate cortex to include the circumstriate and inferior temporal areas. From the inferior temporal areas, in turn, information about these objects appears to be transmitted to subcortical structures in the temporal lobe. We have attempted to map this functional system by means of the 2-deoxyglucose technique, an autoradiographic method for measuring local cerebral glucose utilization (LCGU). To achieve this, we compared LCGU values in a surgically "blinded" hemisphere and in the opposite "seeing" hemisphere in the same monkey while the animal was visually active. Cortically, we found reduced glucose utilization in the blind as compared to the seeing hemisphere not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed areas also included tissue thought to be important in spatial, rather than object, vision.

PHS-6040
(Rev. 10-76)

Subcortically, side-to-side differences were seen in structures associated with processing information about both visual objects and visual space. The results not only provide striking confirmation of an extensive cortical visual system but also help to reveal its full extent and to localize its points of contact with subcortical structures.

PROJECT DESCRIPTION:

Neural structures involved in the processing of visual information have been painstakingly identified through the use of very time-consuming surgical electrophysiological, and anatomical techniques, and yet, the full extent of the visual pathway has probably not been revealed. These techniques have been used to describe a sequential system for processing information about visual objects which begins in the geniculostriate pathway and continues through the prestriate cortex along a corticocortical route to the inferior temporal cortex. Information is then transmitted to limbic and striatal structures, although the precise termination of this system in those areas is unclear. These techniques have also been used to identify another system, specialized to process information about spatial vision, which also depends on a geniculostriate input and a corticocortical pathway, in this case through the prestriate, parietal and prefrontal cortex. In addition, both systems are known to have subcortical inputs to, and projections from, structures in the tectofugal pathway.

Experiment 1

We have attempted to map these functional systems by means of the 2-deoxyglucose technique, an autoradiographic method for measuring cerebral glucose utilization (Kennedy et al., Science, 187: 850, 1975). To achieve this, we prepared monkeys with a unilateral optic tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare values for local cerebral glucose utilization in a "seeing" and "blind" hemisphere within the same animal, and thereby map the visually related areas.

Monkeys in this initial study sat in a primate chair while visual patterns mounted on a drum rotated around their heads. They were injected with a pulse of radioactively-labeled 2-deoxyglucose and monitored over the next 45 minutes to determine the levels of free deoxyglucose in the blood. Most of the radioactively-labeled substance is incorporated into active cells during this period and any extracellular deoxyglucose is of low enough concentration so that its presence does not contaminate the autoradiographs.

In a second study, monkeys with both an optic tract section and split of the forebrain commissures were trained on a visual discrimination task using the hand opposite the blind hemisphere. The task was a pattern discrimination in which a positive stimulus was paired with one of a series of negative stimuli in sequential blocks of trials. Correct responses to the positive stimulus were reinforced with a liquid reward. No reward followed correct responses to the negative stimulus.

In both behavioral situations, reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe, side-to-side differences were seen in lateral and dorsal amygdala, ventral putamen, ventral claustrum, and tail of caudate. Outside this stimulus-processing system and its subcortical projections, asymmetries were also seen in the superior colliculus, inferior and lateral divisions of the pulvinar, and lower bank of the intraparietal sulcus and exposed surface of the inferior parietal lobule. Performance on the discrimination task led to a symmetrical increase of glucose utilization in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking; within the visually related areas, however, the effects of the two different testing situations were substantially the same.

Experiment 2

Portions of each of the visual areas within the cortical pathway serving object vision are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TEO and TE receive their contralateral input via the splenium and anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we attempted to localize and to quantify the contribution to vision made by the commissural systems. To do this we measured rates of local cerebral glucose utilization (LCGU) throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy, and unilateral optic tract section alone. The 2-deoxyglucose method was applied one month postoperatively in awake rhesus monkeys actively viewing visual patterns. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of the two groups.

From the autoradiographs of each brain, representative sections were chosen at 1 mm intervals throughout the extent of the cortical visual pathway. Weighted averages of LCGU for the entire extent of each visual area were then calculated from these sections by means of a computerized image-processing system. In the intact hemisphere there was a progressive decline in LCGU along the cortical visual pathway from a high of 66 in area OC to a low of 47 umoles/100g/min in anterior TE. This sequential decline in the intact hemisphere was the same for both the animals with tract section plus commissurotomy and those with tract section only. There were also no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere (ranging from 40% in OC to 60% in TEO). A difference attributable to visual input via the intact commissures was found in TE, however, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas in animals with tract section only LCGU reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively. These results indicate that commissural inputs contribute more to the visual functions of area TE than to those of any other visual area and that commissural inputs alone may be insufficient to support visual function in areas TEO, OA, and the OC-OB border

Experiment 3

In order to trace the functional development of the visual system, we are conducting studies in infant monkeys similar to those described in the first experiment. Thus far, a series of animals with unilateral optic tract section combined with forebrain commissurotomy have been tested at various ages ranging from 2 days to six months. Preliminary examination of the data suggest that, metabolically, the visual system is almost fully developed by six months, but that prior to this time different parts of the system reach maturity at different times. Quantitative analyses will be undertaken soon to trace the developmental sequence.

Additional studies

The results of these glucose utilization studies in the visual system have provided such striking confirmation of the extent and locus of visually related areas in such a short period of time that we are now undertaking similar experiments in other sensory systems for which, unlike vision, the central processing pathways are virtually unknown. Experiments in progress include one on the olfactory system comparing rodents and primates, and another on the monkey's somatosensory system. The goal is to survey all the sensory systems in order to determine whether each has sensory-limbic and sensory-motor components, whether there are areas of overlap, and whether there are areas that do not fall under the dominant control of one or more sensory modalities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02034-02 LN

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Subcortical Mechanisms Related to Frontal Lobe Functions in the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. E. Rosvold	Chief, Laboratory of Neuropsychology	LN	NIMH
Other:	D. P. Friedman	Staff Fellow	LN	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.9	PROFESSIONAL: 0.7	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The intimate anatomical and biochemical relationship between the caudate nucleus and the substantia nigra suggest that they may subsume similar cognitive functions. It has been established that lesions placed in the caudate nucleus of the rhesus monkey impair performance on delayed alternation performance. However, massive lesions placed in the substantia nigra of a pilot animal had little, if any, effect. It becomes important therefore to establish the generality of this negative finding, and to explore what significance for behavior there is in the biochemical and anatomical similarities in these structures.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The close relationship between the caudate nucleus and the substantia nigra has been demonstrated both anatomically and biochemically. It would be expected, therefore, that these two structures would subsume similar cognitive behavior, yet it has not been shown that they do. The purpose of this project is to examine the possibility that lesions placed in the substantia nigra would, as they do in the caudate nucleus, result in severely impaired performance on the delayed alternation task, a test of cognitive behavior.

Stereotaxic procedures will be used to place the lesions in the substantia nigra of the rhesus monkey with the intention of destroying most of the structure bilaterally. The animals will then be tested on a battery of tests known to reflect impaired function in the caudate nucleus. When the tests have been completed, the brain will be removed and the site of the lesions verified histologically. Although it is difficult to place lesions selectively in the substantia nigra, we have demonstrated in a pilot animal that it is possible to do so. This animal, was, surprisingly, not impaired in performance of the delayed alternation task. It becomes extremely important, therefore, to establish the reliability of this negative finding. Four animals have been prepared on the behavioral tasks and are ready to be operated.

Significance to Mental Health. The monoamines, chemical products of the substantia nigra, raphe nuclei, and the locus ceruleus, have been implicated in mental health and disease. It is not known to what extent they also affect cognitive behavior. It is the purpose of this project to provide this information which presumably could be of diagnostic or therapeutic use.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00471-25 LPP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of Heredity and Environment in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Rosenthal	Chief, Section on Genetics of Mental Illness	LPP NIMH
OTHER:	P. H. Wender	Professor of Psychiatry	University of Utah
	S. S. Kety	Professor of Psychiatry	Harvard Medical School
	S. Nagler	Research Psychologist	Institute for Research on Kibbutz Education (Israel)
	F. Schulsinger	Co-Director	Psychological Institute (Denmark)
	O. W. Quinn	Guest Worker	LPP NIMH

COOPERATING UNITS (if any)

Harvard Medical School, Institute for Research on Kibbutz Education, and Psychological Institute

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION
Section on Genetics of Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	3.1	2.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

PHS-6040
(Rev. 10-76)

Project Description:

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

The analysis of the literature has been reported in a series of papers and in the study of the quadruplets. A textbook which organizes and explicates the literature on the genetics of behavioral disorders and a modified version of this book have been published. Several papers on our adoption studies are now published. The studies indicate that hereditary factors contribute to the development of schizophrenia and to other disorders that we include in a group called the schizophrenia spectrum. Several articles are in preparation, and eventually a monograph will be written on the adoption studies. Data analysis is continuing. One hundred subjects in the Israel study have been examined, and the research findings are currently being analyzed and evaluated. Preliminary findings indicate that offspring of schizophrenics have more neuropathological signs in childhood than do controls. Four subjects have had a breakdown, and all have a schizophrenic parent.

It is significant to bio-medical research and the program of the Institute that we have resolved a chronic, critical problem in psychiatry by demonstrating beyond any reasonable doubt that genetic factors are importantly involved in the etiology of schizophrenia and related mental disorders. Moreover, the evidence thus far suggests that the mode of genetic transmission is polygenic or a dominant single gene with polygenic modifiers.

The proposed course of our project is to examine additional subjects in Denmark with the following goals in mind: (1) to try to discriminate gene carriers and non-gene carriers with respect to personality and test variables; (2) to compare the fate of gene carriers who are reared in the parental home and compared to those reared in adoptive homes; (3) to compare Ss who have a psychotic biological parent but who are reared by nonpsychotics with Ss whose biological parents are not psychotic but who are reared by a psychotic adoptive parent; (4) to find the incidence of schizophrenia spectrum disorder in a random sample of the Danish population. In Israel, our goal is to prepare a volume on our findings and their theoretical implications. During this year we are carrying out reexaminations of this unique sample of subjects.

Publications:

Rimmer, J., Cole, S., Jacobson, B., Kety, S., Rosenthal, D., Schulsinger, F., and Wender, P.: Personal and social characteristics differentiating adoptive relatives of schizophrenics and nonschizophrenics: a preliminary report based on interviews. Compr. Psychiatry 20: 151-158, 1979.

Rosenthal, D.: Foreword. In Shakow, D.: Adaptation in Schizophrenia: The Theory of Segmental Set. New York, John Wiley and Sons, 1979, pp. ix-xi.

Rosenthal, D.: Genetic Factors in Behavioral Disorders. In Roth, M. and Cowie, V. (Eds.): Psychiatry, Genetics and Pathograhpy: A Tribute to Eliot Slater, London, Gaskell Press, 1979, pp. 22-33.

Rosenthal, D.: Genetic Aspects of Schizophrenia. In van Praag, H. M., Lader, M. H., Rafaelsen, O. F., and Sachar, E. J. (Eds.) Handbook of Biological Psychiatry, Vol. III, Brain Mechanisms and Abnormal Behavior. New York, Marcel Dekker, Inc., 1980, pp. 3-34.

In Press:

Rosenthal, D. The Genetic-Environmental Perspective in Psychopathology. In Al-Issa, I. (Ed.): Culture and Psychopathology. Baltimore, University Park Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00472-17 LPP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Investigations of Some Formal Characteristics of Speech

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP	NIMH
OTHER:	D.S. Boomer	Guest Worker	LPP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology & Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research project investigates attention during natural speech perception. Specifically, we are attempting to test the hypothesis that speech perception involves phasic, rather than continuous attention, and is characterized by bursts of cognitive activity at linguistically specified boundaries in the stream of speech. The method involves the measurement of reaction time to irrelevant stimuli--clicks--while the subject is listening to a tape recorded dialogue. If the hypothesis is correct, the responses should be relatively inhibited for those clicks timed to occur during the postulated bursts of cognitive activity at linguistic boundaries. If the fundamental hypothesis can be given empirical support this method will be used to investigate attention deficit in schizophrenia.

Project Description:

The guiding hypothesis of this study growing out of work on listener responses is that listeners, like speakers, process speech in "chunks," or temporal patterns, the chunks being phonemic clauses. If the decoding process operates with phonemic clause speech units then attention to the incoming speech need not be continuous. We hypothesized that attention would be maximally occupied at the end of the phonemic clause and minimally occupied while the clause is being accumulated and stored.

In the present study subjects listen to a rather absorbing tape recorded dialogue in one ear while also attending to an irregular series of clicks presented to the other ear. They respond to each click by depressing a telegraph key as quickly as possible. Reaction times are measured and stored. Half of the clicks are precisely timed to occur at the end, or terminal juncture, of phonemic clauses. Our hypothesis is that the subjects' attention to the cognitive demands of speech decoding will be different during clauses than at the ends of completed clauses, and this difference will be reflected in the mean response latency to the two sets of linguistically-specified clicks.

We found that for right-handed subjects, when speech is presented in the right ear and clicks in the left, reaction times were about 20 msec. faster to clicks at the terminal juncture than to those during the clause (18 of 20 subjects showed this effect). The data suggest that fluctuations of attention do occur during speech perception, but contrary to the original hypothesis speech processing seems to be more active during a clause than at the terminal juncture. During the past year we completed testing of 20 right-handed subjects, but with speech going to the left ear and the clicks to the right ear, and have tested a number of left-handed subjects on the original speech--right ear mode. Most of these subjects also show faster reaction time at the terminal juncture but to a somewhat lesser degree than the original group. However, left-handed males seem not to show any clear effect of click placement. We will continue to try and recruit more left-handed males to complete this group.

Beyond the present normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular attentio to the specification of hemispheric asymmetry in cortical function. It is widel believed that cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically the ability to extract the salient elements of complex stimulus patterns. Since speech is just such a complex pattern and since the hemispheres are asymmetrically specialized for speech, a psycholinguistic approach to the problem of laterality and attention seems to be both neglected and promising.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00476-08 LPP

PERIOD COVERED

TITLE OF PROJECT (80 characters or less)

The Offspring of Schizophrenics: Markers of a Schizophrenic Disposition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.O. Rieder	Research Psychiatrist	LPP NIMH
OTHER:	D. Rosenthal	Research Psychologist	LPP NIMH
	S. Broman	Research Psychologist	DNB NINCDS
	P. Nichols	Research Psychologist	DNB NINCDS
	P.H. Wender	Professor of Psychiatry	Univ. of Utah

COOPERATING UNITS (if any)

Developmental Neurology Branch, NINCDS

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dr. Rieder, the Principal Investigator for this project left NIMH 7/1/79 and this project was terminated as of that date.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00484-20 LPP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Psychophysiological Responsivity and Behavior in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP	NIMH
OTHER:	C. Schooler	Research Psychologist	LSES	NIMH
	M. Buchsbaum	Research Psychiatrist	BPB	NIMH
	D. Murphy	Chief	CNB	NIMH
	D. Van Kammen	Research Psychiatrist	BPB	NIMH
	T.N. Robinson	Guest Worker	LPP	NIMH
	R. Haier	Guest Worker	LPP	NIMH
	S. Haynes	Epidemiologist	HV EP	NHLBI
	L. Siever	Clinical Associate	CNB	NIMH

COOPERATING UNITS (if any)

Laboratory of Socio-enviornmental Studies, Biological Psychiatry Branch, Laboratory of Clinical Science, and Ward 4 East

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general purpose of this project is to investigate the roles of autonomic nervous system activity, attention, and information processing and their interrelationships in the pathology, etiology and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity as assessed by peripheral measures such as skin conductance, heart rate and skin temperature. Subjects with diagnoses of schizophrenia, depression and neurosis are tested under conditions of rest, presentation of tones and performance on reaction time, mental arithmetic, two-flash discrimination or tachistoscopic recognition tasks. Biological mechanisms influencing ANS activity and attention are investigated by testing the effects of drugs and other treatments such as renal dialysis and by correlating these variables with enzyme activity and levels of biogenic amines and their metabolites. Psychological determinants are investigated by correlating the results with personality, mood and personal history questionnaires, by information from interviews, and by the effects of procedural variations.

PHS-6040
(Rev. 10-76)

Project Description:

In a completed study we showed that a pattern of ANS and attentional functioning--high ANS "arousal," small ANS responses particularly to meaningful or demanding stimuli and situations, slow adaptation and habituation and poor attention--characterized unmedicated acute schizophrenics compared to normal controls, and within the schizophrenic group, predicted a poor clinical outcome of a 4-month hospitalization.

Data analysis has begun on another major study of a more heterogeneous group of medication free schizophrenic inpatients and normal controls. (See Z01 MH 00132-05 BPB) The protocol involves ANS recording during two sessions which include rest and stress periods, simple tones and two perceptual-motor tasks: two-flash discrimination and a tachistoscopic recognition procedure. We can test the differential psychophysiological responsiveness of schizophrenics and normals to task difficulty, success and failure, positive and negative reinforcements, etc., as well as being able to separate sensory reception aspects from response aspects of the stimulus complex. In addition to attempting to replicate and extend the previous findings on the relation of ANS activity to improvement we can also relate the ANS variables to the effects of different drugs such as lithium, pimozide, propanalol, GHB, and naloxone and the effects of renal dialysis which are being tried along with placebo on a double-blind basis on these patients. Thus, we can test the feasibility of using ANS variables to aid in the determination of the optimal therapy. In addition, we are testing patients while they are on treatment protocols using a procedure in which ANS variables are recorded during rest, presentation of a series of tones of different intensities and a two-flash threshold determination. These observations may help us determine how ANS changes relate to changes in behavior and to increase our understanding of the central mechanisms involved in of ANS functioning.

Preliminary analysis of the admission data (unmedicated) has partially confirmed the previous study in finding high ANS activation and low reactivity in patients compared to controls. An anomalous finding in the previous study--that skin conductance level, a commonly used index of ANS activation, different from the other indices in being slightly lower in the schizophrenics--was also partially confirmed in that it was quite similar in the two groups.

A study of ANS activity in 7 schizophrenic patients undergoing weekly renal dialysis (active dialysis vs. placebo or sham dialysis) found that on active dialysis the subjects had a significantly larger increase skin conductance level (SCL) to the initial instructions and after the first loud (100 dB) tone in the series, showed an increase rather than a normal decrease in SCL during the series of tones, and had a large HR acceleration to the first loud tone. These results suggest that the active dialysis may have removed something from the system that normally inhibits ANS reactions to novel and/or aversive stimuli.

In normal controls tested on these same procedures we are investigating relationships between ANS activity, personality, behavior and enzyme activity. These subjects (N=97) were given personality, interest, and mood questionnaires and had platelet and plasma monoamine oxidase activity measured. In collaboration with Dr. Schooler (See project Z01 00674-10 LSES) the method of "confirmatory factor analysis," developed for use in social science research, is being used on the psychophysiological and personality data in an attempt to develop error free measurement models of the structures of these systems. This method may lead to causal models of the interrelationships between systems. The PI is collaborating with six other investigators from the U.S. and Europe who have studied the skin conductance orienting response (response to simple stimuli) in schizophrenia, on a paper which casts the results of the numerous studies on this problem into the same format to optimize cross-study comparisons. This paper should help to resolve the apparently conflicting results that have been obtained with this method.

In current studies of attentional processes in unmedicated schizophrenia several methods of assessing attention deficits using reaction time (RT) techniques are being compared: (1) the classical "set" procedure of Shakow which involves variations in the foreperiod in a simple auditory RT paradigm, (2) a choice RT procedure to investigate "sensory dominance." In this method RT to visual and auditory stimuli is measured when the stimuli are predictable, unpredictable or simultaneous (but unpredictably so). We have confirmed previous findings in normals that although RT to tones is faster than RT to lights when the stimuli are predictable, RT to light is faster under unpredictable simultaneous presentation. This is taken to indicate an attentional bias toward visual stimuli or visual dominance, (3) comparison of ipsimodal vs. crossmodal sequences of tones and lights in a simple RT paradigm, plus occasional simultaneous presentation to assess "intersensory facilitation." Simple RT is faster under simultaneous presentation of a tone and light in the context of an unpredictable series presumably because the subject's response is triggered by whichever of the two stimuli he is attending to. Several theoretical models about attention dysfunction in schizophrenia can be tested. Most patients tested have shown visual dominance and intersensory facilitation but more data are needed for quantitative comparisons, and normal controls will be tested.

These procedures have been used in a "high risk" study in collaboration with Dr. Buchsbaum of BPB (Project MH 00035-08 BPB) in which S's were selected for extremely good or poor performance on another test of attention: the continuous performance task. ANS data were also recorded during the "set" procedure. Poor attenders, as defined by the CPT, were found to have increasingly impaired reaction time as the amount of processing required increased from simple (20-25 msec) to choice reaction time (100 msec). This deficit seems due to a problem of shifting attention--from longer to shorter foreperiods in the Shakow procedure or between stimulus modalities in the other procedures. Thus we have found a rather specific attention deficit in normal subjects that appears on several tests and is similar in kind to what has been found in psychopathology. We will relate these findings and the ANS data, when analyzed, to a variety of cognitive, perceptual and personality data that were gathered on these subjects in order to assess possible relationships to psychopathology. A similar study is underway with subjects selected on the basis of pendulum eyetracking.

Psychophysiological assessment of medication-free patients with depression and severe obsessive-compulsive neurosis has begun. Part of the protocol is identical to that currently used with the schizophrenics so that a direct test of the hypothesis of the specificity of ANS patterns for different diagnoses can be tested. Patients will also be tested on tricyclic antidepressants and MAO inhibitors, and normal controls matched on age will be tested. These studies may increase our understanding of the neurobiology of depression and obsessive-compulsive neurosis and of the mechanisms by which the pharmacological treatments affect behavior in addition to our understanding of the mechanisms influencing ANS activity.

Data analysis has been completed on one of two studies of the effects of stress on concomitant changes in skin conductance, heart rate, skin temperature, blood pressure and plasma norepinephrine indices of sympathetic nervous system activity. We studied middle aged men screened on a measure of "coronary prone personality" (Type A), and the above variables were assessed during a succession of rest and stress periods which included a 5 min. stand, a mental arithmetic task and the "Type A, B" interview. The variables measured differed in their response to stress for the group as a whole. Norepinephrine increased markedly during standing but did not increase appreciably to the psychological stresses while all other variables showed marked and significant increases in these periods (usually $\underline{p} < .001$). Type B men showed significantly larger increases in heart rate, and norepinephrine than type A men during the stand and also had higher resting levels on these measures. Type B men also had a significantly larger decrease in skin temperature during the arithmetic stress but type A men had marginally significant greater responses on blood pressure and skin conductance variables. On a mood scale type A men showed a trend to give higher self ratings of depression during the stresses ($\underline{p} < .06$). This study is consistent with the increasing evidence that the sympathetic nervous system does not act as a unit, but that the type of situation and personality of the subject interact to produce different response patterns. We hope to extend this research in such ways as varying the opportunity for "active coping" during stress periods and analyzing plasma for other substances such as cortisol and β-endorphins.

Publications

Zahn, T.P.: Predicting outcome from measures of attention and autonomic functioning. In Baxter, C. and Melnechuk, T. (Eds.): Perspectives in Schizophrenia Research, Raven Press, in press.

Zahn, T.P. Carpenter, W.T., Jr., and McGlashan, T.H.: Autonomic nervous system activity in acute schizophrenia: I. Method and comparison with normal controls. Arch. Gen. Psychiat., in press.

Zahn, T.P., Carpenter, W.T., Jr., and McGlashan, T.H.: Autonomic nervous system activity in acute schizophrenia: II. Relationships to short term prognosis and clinical state. Arch. Gen. Psychiat., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00486-08 LPP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Psychophysiological Concomitants of Minimal Brain Dysfunction in Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP	NIMH
OTHER:	J.L. Rapoport	Medical Officer	BPB	NIMH
	R.Elkins	Clinical Associate	BPB	NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA. NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many investigators believe that the autonomic nervous system (ANS) may be involved in hyperactivity (HA) in children. The objectives of our studies are to investigate differences in autonomic functioning between HA and normal children by means of peripheral indicators such as skin conductance, heart rate and skin temperature, and to assess the effects of the drugs on both autonomic activity and on task performance which depends on attention such as reaction time. The hypothesis that HA and normal children respond differently to stimulant drugs has been tested in a study of the effects of d-amphetamine on autonomic activity and attention in 6-13 year-old normal and HA boys. Male adults have been tested on the same protocol to test for age differences in drug effects. Studies have been done on the acute and chronic effects of caffeine on these measures in boys and a similar study has begun on normal men.

Project Description

The general purpose of this project is to investigate the role of autonomic nervous system (ANS) activity and attention in hyperactivity in children and to study the effects of stimulant drugs on these processes in hyperactive and normal children and in normal adults.

Last year we reported a study (with J. Rapoport of BPB, Z01 MH 00486-08) comparing the effects of d-amphetamine in normal and hyperactive boys which tested the widely held hypothesis that the effects of this drug are atypical in hyperactive boys. Both groups showed reduced motor activity, improved attention and reduction in impulsive behavior after a .5 mg/kg dose of amphetamine compared to placebo as well as increased heart rate slowing in anticipation of reaction time stimuli, a response associated with attention. The drug produced a reduction in skin conductance activity in both groups, although not in the same part of the session for the two groups. In general, the effects of the drug were similar in the two groups and consistent with the literature on drug effects in hyperactive children. Thus hyperactive children do not respond atypically to amphetamine.

We have completed the analyses of a similar study testing the effects of .25 and .5 mg/kg doses of amphetamine on normal men. On just the low dose of the drug the subjects had decreased motor activity and some improvement in reaction time. Autonomic effects were more marked on the high dose. The drug produced higher indices of arousal, slow habituation, less cardiac deceleration in reaction time foreperiods and a general reduction in skin conductance activity. This reduction was disproportionately greater in the case of meaningful or demanding stimuli and situations than in the less demanding portions of the session. The response to amphetamine in the men can be conceptualized as a decrease in the specificity of ANS reactions to imperative or demanding stimuli and situations while in the boys there was an increase in this specificity. The ANS effects of amphetamine on men are remarkably similar to those obtained in comparing drug-free schizophrenics with controls (see Z01 MH 00484-20 (LPP) and suggest a common mechanism may be involved in both reactions.

New studies are testing the effects of caffeine in boys and men using the same experimental procedures as were used in the amphetamine studies. The objectives of these studies is to compare the effects of these two "stimulant" drugs and, since caffeine has been reported to be a competitive inhibitor of the benzodiazapine receptor, to evaluate it as a possible pharmacological model of anxiety.

In one study the effects of single doses of 3 mg/kg and 10 mg/kg of caffeine and placebo were compared in 19 boys. Marked increases in two electrodermal arousal measures--frequency of spontaneous skin conductance responses and skin conductance level--after caffeine ingestion, but heart rate decreased. Caffeine also increased the frequency of skin conductance responses to simple tones and stimuli in the reaction time task, produced an increase in motor activity and improved reaction time. The results show a partial concordance with expectations based on previous studies of clinical anxiety and effects of anti-anxiety drugs.

In a second experiment, boys selected on the basis of being high or low caffeine users were tested at baseline and after two weeks of a double blind regimen of caffeine or placebo. The most consistent effects were significant increases in spontaneous skin conductance responses and in cardiac decelerations in the reaction time foreperiods for the low use group. The general lack of strong effects of caffeine in this study suggests that tolerance of its effects on ANS activity develops over a two week period. However, the two caffeine use groups differed at baseline in that the high use group gave consistently smaller skin conductance responses. This could be due to a change in receptor sensitivity due to prolonged caffeine use or reflect a difference in the personalities of high and low caffeine users.

A study of acute effects of caffeine in men who are either high or low users is underway.

These studies are significant for biomedical research and the program of the institute in several ways. First, the study of ANS changes after drug administration may help elucidate the mode of action of the clinical effects. For example, the more "focussed" ANS activity in children and less focussed ANS activity in adults produced by amphetamine can be seen as consistent with the improvement in attention seen in children and the adverse effects in adults from amphetamine abuse and the exacerbation of symptoms in schizophrenia by amphetamine. Second, since the pharmacological effects of these drugs are somewhat understood, these studies can elucidate the mechanisms of ANS activity and help to interpret the ANS findings on clinical populations. Third, amphetamine abuse is a public health problem and caffeine abuse may be one. Further understanding of the biological and psychological effects of these drugs may help to deal with these problems.

Publications:

Zahn, T.P., Rapoport, J.L., and Thompson, C.L. Autonomic and behavioral effects of dextroamphetamine and placebo in hyperactive and normal prepubertal boys. J. Abnorm. Child Psychol. 8:145-160, 1980.

Rapoport, J.L., Buchsbaum, M.S., Weingartner, H., Zahn, T.P., Ludlow, C., Mikkelsen, E.J., Langer, D., and Bunney, W.E., Jr. Dextroamphetamine: Cognitive and behavioral effects in normal and hyperactive children and normal adults. Arch. Gen. Psychiat., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00487-08 LPP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Autonomic Functioning in MZ and DZ Twins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP	NIMH	
OTHER:	M. Buchsbaum	Research Psychiatrist	BPB	NIMH	
	A. Sostek	Post-Doctoral Fellow	LCP	SMRC	NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, DCBR, NIMH

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No work on this project was done in the past year. It will be discontinued as a separate project and any future developments will be reported in Z01 MH 00484 LPP

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00488-15 LPP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Individual Differences in Survival and Reproduction Among Old Colony Mennonites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Allen	Guest Worker	LPP NIMH
OTHER:	C. W. Redekop	Professor of Sociology and Anthropology	Univ. of Waterloo and Conrad Grebel College

COOPERATING UNITS (if any)

University of Waterloo and Conrad Grebel College

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was undertaken to seek evidence of natural selection with respect to human behavioral characteristics. The population chosen consisted of about 13,000 German-speaking Mennonites whose culture resembles in many respects that of pre-industrial European peasants.

A census-survey was made in 1967 and vital records of the church were copied. For 569 families in the file, data include complete vital records and factor scores on several dimensions of social and economic behavior. Lengthy editing processes have now been completed and final analysis is being planned. This will first, specify parameters of survival and reproduction; second, explore interrelations among vital events such as effect of infant mortality on birth intervals; third, examine the effects on fertility of such variables as wealth, medical care, and social status.

Project Description:

This investigation was undertaken in 1965, mainly to seek evidence of natural selection with respect to human behavioral characteristics. The study of selection in a pre-industrial type of European culture may indicate some of the influences that have produced present genetic tendencies and would draw a base line for future studies of selection under modern conditions. A secondary objective was to define natural patterns of marriage and reproduction that might have a bearing on the growth and regulation of human populations.

The population under study consists of about 13,000 German-speaking Mennonites in a politically integrated cluster of 46 villages in northern Mexico. These people were selected because of their simple and uniform culture insulated from mass communication media and because of their apparently unrestricted fertility. Also important was the fact that, unlike some similar religious isolates, this society supports a considerable degree of economic competition.

Professor Calvin Redekop, of the Department of Sociology and Anthropology at Conrad Grebel College and the University of Waterloo, has assisted in the planning and execution of the study. He speaks the Low German dialect of these people and has lived among them.

Information was gathered by means of a combined census and survey that reached a third of the population. This was supplemented with photographs of all vital records kept by the church, a public list of farmers and their holdings, a list of all village and church leaders in the 40-year history of the population, and records of Mennonite births in local hospitals.

Data on births, marriages and deaths, including complete reproductive histories, were cross-edited between church and census records with the elimination of many errors and omissions in both sources. This editing was completed at the end of 1979. On 569 families, we now have complete vital records, the results of the socio-economic survey, and factor scores on several dimensions of social and economic behavior.

Analysis will be directed to at least the following three subjects:

1. Life table analysis of female fertility.
2. Effects on male and female fertility of age at marriage, infant deaths, and widowhood/remarriage.
3. Correlation of fertility with socio-economic variables including wealth, medical care, and social status.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00489-23 LPP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Variables Affecting Twin Birth Frequencies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. Allen Guest Worker LPP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Significant variations in the twinning rate can be observed both in the long term and in the short term. Attempts to explain such variation have been based variously on coital frequency, declining sperm counts, a fertility advantage in twin-prone women, variation in early loss of embryos, and other phenomena.

Evidence is being reviewed, including recent vital statistics and current views of the physiology of reproduction, in the expectation that a decision can be made as to the probable explanation of twinning rate variations.

PHS-6040
(Rev. 10-76)

Project Description:

This project, begun in 1957, has had three phases. In the first, U.S. birth statistics were used to document variations in twinning rates over space and time. This led to new support for an old hypothesis; namely, that twin-prone women are more fertile than others. If true, this would explain much of the variation in the twinning rate. A quantitative estimate was obtained for the fertility advantage required of these women to explain the observations.

In the second phase, data were contributed by the Perinatal Research Branch, NINCDS, on 50,000 births studied prospectively. Analysis of these births, together with the complete reproductive histories of the mothers, permitted a rather decisive exclusion of the hypothesis of increased fertility in mothers of dizygotic twins.

The third phase is an attempt to resolve the paradoxical evidence for and against elevated fertility in twin-prone women. The current state of knowledge has been reviewed concerning physiology of ovulation and fertilization, survival and loss of early embryos, American patterns of fertility and birth control in recent years, and the latest changes in the twinning rate. Consideration has been given to a serious criticism sometimes aimed at the usual method of statistically apportioning twin births between monozygotic and dizygotic types, known as Weinberg's difference method.

Findings:

Support was found for Weinberg's method. If the criticism is correct, the number of MZ births estimated in any age or parity group by Weinberg's method must be reduced by a quantity equal to about 20% of the opposite-sex twins. When this is done, the usual straight line describing the relation of MZ twinning to maternal age is replaced by a concave curve, mirror-imaging that of opposite-sex twins. This suggests that the negative adjustment is wrong.

A more interesting finding, but one whose interpretation remains a speculation, is that several sources of evidence for the (discredited) fertility advantage of twin-prone women yield quantitatively incompatible estimates of the advantage.

An explanation may be found in the fact that these sources derive from samples of women in different social and psychological circumstances: out-of-wedlock conceptions, first marital conceptions, and first conceptions after the war. These circumstances could have different psychological effects on pituitary function; hence on release of FSH, which probably controls double ovulation. The different apparent levels of fertility in mothers of twins would then be explained by different rates of twinning. Independent support for this idea is found in studies of women who have just terminated oral contraception.

Publications:

Allen, G. and Hurbec, Z.: Twin concordance: a more general model. Acta Genet. Med. Gemellol. 28: 3-13, 1979.

Allen, G.: Holzinger's H_c revised. Acta genet. Med. Gemellol. 28: 161-164, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00491-04 LPP

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Personality factors and psychophysiological responses to changing stimulus input.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP	NIMH
Other:	T.N. Robinson, Jr.	Guest Worker	LPP	NIMH

Professional Personnel - 10/1/78 through 9/30/79

PI:	T.N. Robinson, Jr.	Staff Fellow	LPP	NIMH
Other:	T.P. Zahn	Research Psychologist	LPP	NIMH
	R.O. Rieder	Research Psychiatrist	LPP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to investigate relationships between differences in personality, sensory thresholds, and autonomic nervous system (ANS) activity in normal humans and to study racial differences in ANS activity. Bilateral skin conductance, heart rate, vasomotor activity and respiration have been recorded in two sessions in which constant and variable intensity tones and lights are presented and auditory and two flash thresholds determined by methods which permit signal detection analyses. Several standardized personality tests were also given. The procedures allow determination of the effects of stimulus intensity and heteromodal stimulation on ANS activity. The protocol allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity and personality, some of which have implications for the etiology of psychopathology.

Project Description

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion", "sensation seeking", and "field dependence", each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships between personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., overreactive to weak stimulation and underreactive to strong stimulation--"transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, and ANS activity in normal humans. Other objectives are to assess racial differences in ANS activity and in its relationships to the other variables in the study and to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality.

Over 180 normal volunteers have been assessed on several personality dimensions including the Eysenck scale of extraversion, neuroticism and psychoticism, field dependence, sensation seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance and given tests of ANS and sensory functioning in two separate sessions as described earlier.

Results to date show no support for the prediction that introverts should show a transmarginal inhibition. Contrary to this prediction introverts compared to extraverts had large ANS reactions to high intensity auditory stimulation. Nor do the predictions of these theories with respect to the interrelationships between personality, ANS "arousal", and sensory thresholds hold up since we found that subjects giving evidence of higher ANS "arousal" showed high thresholds and low sensitivity and subjects who, from the personality measures would be expected to have "strong" nervous systems showed greater sensory sensivity. However, a limited confirmation of the predictive validity of the "psychoticism" measure was found. Subjects scoring high and low on this scale differed in the direction of the relationships between ANS arousal and two-flash threshold and sensitivity. These are similar to results found in comparing schizophrenic and normal subjects.

Further data analysis of this large data base is continuing. In addition to further tests of the personality theories, racial differences and laterality differences will be studied.

Publications:

Robinson, T.N., Jr., and Zahn, T.P.: Covariation of two-flash threshold and autonomic arousal for high and low scorers on a measure of psychoticism. Br. J. Soc. Clin. Psychol. 18:431-441, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HHS ~~HEALTH, EDUCATION, AND WELFARE~~ PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00492-04 LPP

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Relationships among personality, biochemical and psychophysiological variables in college students.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Monte S. Buchsbaum	Chief, Psychophysiology Section	BPB	NIMH
Other:	Dennis L. Murphy	Chief, Neuropharmacology Branch	LCS	NIMH

(PI changed from R. Haier 10/1/80)

COOPERATING UNITS (if any)
Biological Psychiatry Branch, DCBR, NIMH
Laboratory of Clinical Science, DCBR, NIMH

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: .5	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been inactive during this reporting period.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00495-04 LPP

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Psychobiology of Cognitive Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been designed to explore the psychobiology of cognition in man. This research attempts to interrelate psychological and biological determinants of various components of cognition such as the specific and discrete psychobiological mechanisms that define the acquisition, processing, consolidation and retrieval of experience. Experiments are designed to examine the changes in cognitive processes in response to pharmacological and behavioral manipulations, as well as in patient groups with impairment in cognition. Some of these studies have examined whether drugs that enhance aspects of cognition would reverse cognitive disturbances in patients. Findings include the cognitive changes (encoding, memory, retrieval) that occur in response to drug treatments that alter the activity of cholinergic and noradrenic central nervous system activity in patients with various psychiatric disorders and normal controls. Cognitive changes produced by neuropeptides in depressed patients and controls have also been evaluated. The structure and determinants of cognitive impairments are examined and contrasted in patients suffering from various forms of dementia.

These include Alzheimer's disease, senile dementia, Huntington's disease, progressive supranuclear palsy, and Korsakoff's disease. The psychobiological mechanisms of cognitive failures in mood disorders, schizophrenia, hyperactivity, and learning disabilities are also studied in terms of both implications for clinical implications and to further our understanding of the structure of cognitive mechanisms in man.

Project Description:

The research projects reviewed here are all concerned with the psychobiology of cognitive processes in man. The projects have been designed to explore together the psychological and biological determinants of various aspects of cognitive processes and their interrelationships. Studies have examined the psychobiological processes that define the processing, learning, and storage of information, new experience, how processed events are altered, or elaborated in memory, the consolidation and retention of information, and the mechanisms that are involved in retrieval of stored information. Some of the components of cognitive processes that have been examined include attentional determinants, aspects of short-term memory, the consolidation of information in long-term memory, the kinds of state or trait specific cognitive strategies used in the elaboration and transformation of information, organization in memory, "effortful" vs. automatic cognitive processes, and the kinds of strategies that subjects use to retrieve experience that they have stored in memory.

These research efforts have utilized two broadly conceptualized strategies for defining the psychobiology of cognitive processes. One is to contrast the effects of different treatment strategies on different components of cognition, such as on information storage, consolidation, encoding and retrieval. These studies include attempts to systematically alter cognition through 1) pharmacological manipulations, such as cholinergic drugs, noradrenergic drugs, abused drugs (alcohol, marijuana), central nervous system depressants, neuropeptides, and 2) by behavioral manipulations that alter, arousal/activation, stimulus attributes (altering encodability) and types of stimulus processing strategies subjects use to process information. These studies are carried out in normal man, as well as in patient groups with different forms of psychopathology. Would different kinds of pharmacological or behavioral manipulations of cognition lead to different forms of enhanced or disrupted cognition? Contrasting manipulations of different systems in the CNS producing different cognitive effects would help provide a picture of structure of the psychobiology of cognition. The rationale for the alternative strategy for researching the psychobiology of cognition is to contrast the different forms of cognitive failures seen in different psychiatric and neurological syndromes. Would the disrupted cognition seen in some psychopathological states be qualitatively and quantitatively different and related to specific changes in central nervous system activity? For example, how are the amnesic/cognitive impairments seen in Huntington's disease, Korsakoff syndrome, Alzheimer's disorder, different, and how might differences be an expression of the specificity of central nervous system involvement in each of these disorders? In some instances, the possibility of discriminating between the form of the cognitive impairment is necessary for both adequate diagnosis and then effective treatment, such as the cognitive disruptions that are part of depression as opposed to that produced by a progressive dementia. Frequently, depression is an integral part of a progressive dementia, and the cognitive impairment that is evident is a joint product of both of these forms of disorder. Some studies that are part of this project have also investigated the therapeutic potential of various psychoactive drugs and behavioral treatments as these might attenuate or reverse the cognitive disruptions seen in various forms of dementia, hyperactivity and learning disability syndromes in children, depression, mania and the schizophrenias. Each of these studies that make up this research project can be seen as clinically relevant, as well as pertinent for understanding unimpaired cognitive processes.

In summary, all of the research studies that are part of this project are involved in defining the discrete psychobiological components of cognitive processes that are involved in the appreciation, storage, retention, and retrieval of experience. The studies are concerned both with the bases of cognitive processes as well as clinical studies of disordered cognition.

1. Behaviorally defined mechanisms that alter components of cognition.

Characteristics of stimuli such as a) imagery, b) emotional arousing attributes of stimuli, c) information presentation rate, d) mode of processing, e) language vs. pattern information, f) types of learning have been studied in relation to its effects on acquisition (learning), strength of learning, retention, and components of information retrieval. These studies have examined these factors in normal controls, as well as in patient groups (depressed patients, hyperactive children, learning disabled, patients suffering from various forms of dementia). Some of the issues raised in these studies include the following: How might aspects of information processing alter the attentional, short-term memory, encodability, retention, and retrieval of information? Would different forms of psychological manipulations systematically alter different aspects or components of cognitive processes? Do patients who demonstrate failures to learn and remember do so because of disruptions in some, but not all of these component cognitive processes, and can manipulations of some characteristics of information processing change these disruptions in cognition? Recent studies have also begun to explore "effortful" vs. "automatic" information processing and how these are altered under different motivation/arousal conditions. This approach to cognition has been used to define the effects of depression on cognition, determinants of cognitive failures in the learning-disabled child, and drug-altered changes in cognition (see below).

2. Pharmacological alterations (enhancement and disruption) of cognitive processes.

These studies have contrasted the cognitive enhancing effects of cholinergic agents such as arecoline, physostigmine, lecithin treatment with those of amphetamine in both normal subjects, as well as in patients (hyperactive children, Alzheimer's disorder, depressed patients). These studies have also included investigations of cognitive response to neuropeptides. Would different kinds of psychoactive drug treatments that produce qualitatively different central nervous system responses produce systematically different changes in cognitive processing? That is, would enhancements or disruptions of cognition occur through different psychobiological mechanisms, and might the cognitive response to different drugs make such a pattern discernible?

In some instances, drugs that might disrupt aspects of the acquisition of information, may enhance some other stage of cognitive processing (e.g., alcohol administered prior to, or just after acquisition). These stage and process-specific effects of drugs that alter cognition can be used to better define a psychobiology of information processing in man. This research has also involved attempts to find clinically useful strategies for altering disrupted cognition in man.

3. State-dependent learning.

This area of research provides a useful framework for exploring:

a) The qualitatively unique manner in which events are stored (encoded and retrieved from memory).

b) Studies of disturbances in mood state and how these define mood specific strategies for processing experience and remembering past events in memory.

c) Qualitative changes in cognition in response to psychoactive drugs.

d) Contextual factors as determinants for defining the nature of trace events in memory.

e) Individual differences in susceptibility to state dependent or dissociative mood/drug effects.

4. Cognition and mood.

Studies have included research of mood-related changes in: 1) the brain lateralization of cognitive functions; 2) arousal and activation and its role in information processing; and 3) the encoding and retrieval of events in normal mood and while depressed or manic. This research has examined how patients with disturbances in mood, process information in a mood-state specific manner. In addition, this research has begun to examine mood-related changes following psychoactive drug treatment and its interactive role in altering cognitive processes. Other research has explored the degree to which effortful processing of information is compromised as a motivation-related determinant of thinking in depressed patients.

5. Memory Consolidation.

This research has focused on the psychobiological events that follow the acquisition (storage) of information and well before processed information is to be retrieved from memory. Studies in both normal subjects and patients have examined the form and strength of stored information in memory and the processes that might further sustain, enhance, or disrupt stored trace events that are already part of memory. The rate of decay of information, the susceptibility of information to interference and biological factors, may be important determinants in defining what is available and accessible in recall, once information has been stored in memory. Disruptions in consolidation may contribute to the cognitive failures in the dementias.

Drugs that disrupt memory and learning may do so by altering biological operations that succeed acquisition or learning. Likewise, drugs e.g., neuropeptides may enhance aspects of learning and memory by facilitating the consolidation of learned information.

6. Cognitive disruptions in the dementias.

Studies have examined and contrasted the specific mechanisms and structure that defines cognitive failures in different neuropsychiatric populations. Studies of components of cognition that are affected in Huntington's disease have been systematically explored and contrasted to cognitive processing in Korsakoff's psychosis, Alzheimer's disorder, and other neuropsychiatric disorders. These studies have attempted to discover whether much of the cognitive failures seen in these syndromes may be due to encoding (acquisition failures), unreliable access to information which is in memory, interference factors, and disruptions in consolidation. These studies have attempted to uncover the mechanisms or determinants of the cognitive failures in different forms of dementia. Understanding the structure of such impairments is considered essential in conceptualizing and evaluating therapeutic drug trials that might attenuate these cognitive impairments. These studies have lead to trials which have explored how various psychoactive drugs might prove useful in reversing aspects of the cognitive changes that define a particular dementia and uncovering what underlying psychological mechanisms may be disrupted in each of these syndromes.

7. Mechanisms of cognitive impairments that determine forms of learning disabilities.

Studies have been designed which investigate a) forms and incidence of various kinds of learning-disabilities in children, b) the descriptive nature of the learning disabilities in these children; c) cognitive and process determinants that produce impaired learning in these children, and d) potential strategies for their remediation.

Methods

A large variety of cognitive strategies have been designed to explore the psychobiological components of cognition in the studies that are part of this project. These strategies have included modified forms of current methods used in human information processing research, as well as newly-developed tools that might more adequately examine determinants of cognition in clinical studies and those assessing cognitive drug effects. These strategies have been developed in a number of studies and include measures and manipulations of:

a) organization of information (sorting of information, labeling or coding clusters of events such as words, pattern information varying in structure);

b) alterations in the informational context for processing information;

c) stimulus attributes (imagery, emotional properties, frequency, mode of presentation);

d) various forms of learning and recall (free recall, prompted free recall, recognition memory, cued recall, serial learning, paired associates learning);

e) processing time and type of presentation of information;

f) type of processing strategy (processing on the basis of meaning or sound properties);

g) immediate vs. delayed recall of information with or without rehearsal of stored information;

h) presentation of language vs. pattern information to left vs. right hemisphere (methods used to investigate lateralization);

i) rapid (tachistoscopic) presentation of information;

j) measurement and manipulation of different forms of retrieval of information in memory, including forms of free recall, prompted or cued recall, recognition memory, method of "savings;"

k) measures of very long-term memory retrieval;

l) assessment of effortful and automatic cognitive processes;

m) methods of assessing arousal and motivation.

Major Findings: Studies of psychobiological determinants of cognition in unimpaired subjects.

In a number of experiments we have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and its retrieval. Arecoline, physostigmine, and lecithin treatments produce enhancements in the storage and retrieval of information. Scopolamine treatments which disrupt cholinergic activity, produce an impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine-induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated patients with Huntington's disease.

In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in an undrugged state, and afterwards are treated with arecoline, there is a comparable facilitation of information later recalled, when compared to learning and recall that occurs under arecoline treatment conditions.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input processing under drug state conditions. Unlike cholinergic manipulations, amphetamine appears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (size of the pool of trace events in memory). In a series of studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition in depressed patients, hyperactive children, normal children, and normal adults. Amphetamine also induces a change in state which serves as a state-specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state-dependent learning and state-dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine, and the cognitive disrupting effects produced by scopolamine vs. lithium has served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

Alcohol, when administered after the processing of information, produces an enhancement in recall when tested in the unintoxicated state. This paradoxical cognitive facilitating effect of alcohol, administered during a consolidation phase of memory, highlights the differentiated mechanisms and components that make up information processing, memory, learning, and retrieval.

Some behavioral strategies have also been used to investigate the specificity of cognitive processes as these might be involved either separately or together in the acquisition, elaboration and amplification of trace maintenance in memory and retrieval. The emotional properties of stimuli seem to amplify or strengthen what is learned or stored in memory in a manner similar to that produced by drug treatments such as amphetamine. Reinforcement following information processing also acts to strengthen events already in memory storage. Other behavioral determinants, such as word imagery, presentation rate, familiarity, stimulus structure, appear to alter how much is learned, rather than the strength of learning, in a manner similar to the effects of increased cholinergic activity.

Recent findings from behavioral studies of cognitive processes in unimpaired individuals have differentiated two kinds of cognitive operations. One kind requires effortful processing and results in effective encoding of events and the establishment of viable memory traces. The other kind of process appears to be relatively "automatic" requiring little effort, and is not easily disrupted in man. The mechanisms of these processes appear to be different. Drugs that alter one kind of processing do not seem to alter the other kind of cognitive operation [e.g., alcohol impairs recall of items (effortful processing), but not their frequency of occurrence (automatic processing)]. This scheme for describing the structure of cognition (thinking, learning, remembering in man will be investigated further).

Clinical studies of the psychobiological determinants of cognitive processes.

Clinical studies have been designed to investigate the specificity of changes in components of cognitive processes in adults with disturbances in mood, schizophrenic patients, hyperactive and learning-disabled children, and patients with different forms of dementia. These studies have contrasted the different forms of disrupted cognition in Huntington's disease (HD), Korsakoff's psychosis, Alzheimer's disorder, and other neurological disorders.

Drug strategies for altering cognition in impaired patients.

The effects of cholinergic agents in altering cognition in man are systematically inconsistent across individuals. For example, slow learners benefit more (learn more effectively) following administration of cholinergic drugs, while "good" learners are less affected by these drugs.

In therapeutic trials in patients with progressive idiopathic dementias (e.g., Alzheimer's disease), we have observed a small but reliable cognitive facilitation. This has been evident following arecoline administration and in response to the administration of a combination of cholinergic substances. This work is being expanded and capitalizes on two other sets of findings, a) one that defines the mechanisms of memory failure in senile dementia, and b) the specific effects of neuropeptides on cognition.

Amphetamine drug trials in children that have explored state-dependent learning have demonstrated stiking individual differences in dissociative effects. Fifteen children have been studied under four different experimental conditions including, a) learn under drug condition (LD), recall under drug condition (RD), b) or learn following placebo treatment (LP), recall following placebo treatment (RP), both of which are congruent learning recall conditions in contrast to, c) LD, RP, or d) LP, RD, both of which are disparate learning, recall conditions. Somewhat more than half of these children show clear dissociative effects, such that when learning occurs under one condition, e.g., following amphetamine treatment, but memory is tested in the undrugged state, then memory appears to "fail," particularly for poorly processed items, or those that are recalled without benefit of cues or prompts. These findings point to individual differences in the amphetamine response with respect to the form of the cognitive effects in hyperactive children. These dissociative effects also have clear and important implications for the classroom functioning of stimulant-drug-treated hyperactive children.

Studies that define cognitive changes in clinical populations.

HD patients fail to consistently remember information which is, nevertheless, in memory store. They also demonstrate retrieval failures which are due to inadequate encoding of information during acquisition. They fail to use slow presentation rate, semantic encoding, imagery, and other attributes of stimuli to more effectively and elaborately encode information. Some drug and behavioral treatments may prove helpful to normalize this encoding disruption.

Alzheimer's patients also fail to "appreciate," learn, and retrieve experiences. These patients have access to appropriate semantic structures in memory but fail to use these in organizing experiences so that they might prove memorable.

In contrast to HD patients, Alzheimer's patients appear to show a small but measurable cognitive response (improvement) following cholinergic and amphetamine treatment. Recall of organized information is most facilitated while rote-like learning seems unaltered. It is, as yet, uncertain whether the cholinergic response is qualitatively different from that seen following amphetamine treatment. These patients have also been treated continuously with a cholinergic drug, have appeared to show a temporary improvement in cognitive functions, but this enhancement is not a sustained drug response. Strategies for defining cognitive changes in Alzheimer's disorder, and potential treatment strategies are relevant issues in the study of normal aging processes.

Recent studies testing the cognitive changes that follow treatment with vasopressin-like compounds (DDAVP) may provide us with some new strategies in the treatment of dementias. We have shown that DDAVP produces a marked and sustained cognitive enhancement in depressed patients, as well as in normal controls. The form of that cognitive enhancement appears to act through altering organizational mechanisms which may be particularly relevant for the kinds of disruptions in cognition seen in Alzheimer's patients. Studies of the structure of cognitive changes in response to neuropeptide treatment should prove helpful in defining biological mechanisms that are important for those cognitive processes involved in the organization and elaboration of information in memory.

Recent completed cognitive studies of depression have shown that these patients demonstrate learning memory failures when they are required to organize information but show no such deficit when organization is present. That is, when information is presented in an organized fashion, then depression-related cognitive deficits are not apparent. In some respects, their cognitive disruption is similar to that seen in Alzheimer's disorder. These patients also demonstrate qualitative changes in how they encode events and the strategies by which they recollect previously learned experiences (state-dependent learning). Some of these effects may be related to alterations in lateralization of cognitive processes.

Studies of cognitive processes in children have demonstrated some of the specificity of cognitive disturbances that are apparent in hyperactive and learning-disabled children. Hyperactive children appear to learn (store) about the same amount of information as normal controls (under experimental conditions). However, unlike controls, retrieval of that information is highly dependent on the presence of strong environmental cues or prompts at the time remembering takes place. Both normal and hyperactive children respond with enhanced cognition following amphetamine treatment. However, those aspects of cognition that are enhanced in these different children are qualitatively different. The facilitation of cognitive functions in the hyperactive child is one which would not readily translate into more effective school performance, since amphetamine increases learning and recall of superficial characteristics of information processing. This is in contrast to an amphetamine-induced increase in learning and recall of meaningfully processed information in the normal child. It, therefore, appears useful to integrate behavioral retraining strategies along with stimulant treatment to effectively help the hyperactive child.

An integrative system for exploring the psychobiology of cognitive processes.

Ongoing studies have yielded findings which permit us to examine how, a) specific aspects of cognition are altered by drugs that affect activity in different parts of the central nervous systems, and, b) different forms of neuropathology and psychopathology lead to different alterations in the cognitive processes that normally would lead to successful appreciation, acquisition and retrieval of experience. The extensive findings from the studies described here have suggested a pattern to the pharmacological, behavioral, and clinical finding that suggests that distinctive psychobiological mechanisms are involved in the organization encoding, consistent retrieval, and consolidation of information in memory. Different behavioral-neurochemical determinants may alter the strength of trace events in memory, their accessibility, and the amount of stored experience in memory.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00496-04 LPP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Animal Models of Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S.M. Anderson	Staff Fellow	LPP, NIMH

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Laboratory of Psychology and Psychopathology

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was terminated as of 9/19/79.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00499-02 LPP

PERIOD COVERED

TITLE OF PROJECT (80 characters or less)

Biological and Family Studies of Chronic Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dr. Rieder, the Principal Investigator for this project left NIMH 7/1/79 and this project was terminated as of that date.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00500-01 LPP

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cognitive and Perceptual Changes in Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the cognitive and perceptual changes which are present in, and characteristic of, major affective illness. The present investigation comprises eight separate ongoing studies: (1) psychomotor and psychosensory symptoms in affective illness, patients with complex partial seizures, and patient controls; (2) perception and recall of emotional and neutral stimuli in depression; (3) hypothesis testing in depression; (4) Luria neuropsychological evaluation of depression, schizophrenia, and Alzheimer's disease; (5) cognitive and mood response to epinephrine in normal and depressed subjects; (6) lateralized hemispheric function in depression; (7) Halstead category test performance in depression; (8) visual and olfactory perceptual changes in depression.

Project Description

The premise of this project is that there are, in addition to the well known mood changes, important alterations in perceptual and cognitive processes in major affective illness. Such symptomatology may have important implications for the pathophysiology of affective disease, and may provide significant contributions to delineating clinically and prognostically homogeneous subtypes of this condition. The present study is composed of eight investigations, looking at various aspects of the problem. They are aimed at documenting the nature and extent of cognitive and perceptual changes at the clinical level, and of investigating the degree and structure of such deficits in the laboratory. Below is a summary of the current studies within this project.

1. Psychomotor and psychosensory symptoms in affective illness. A wide range of behavioral and perceptual manifestations, many with localizing significance in the brain, have been described as concomitants of complex partial seizures. The literature and clinical observation suggest that there may be areas of overlap between these phenomena and symptoms of major affective illness. The purpose of the present study is to investigate the nature and degree of such overlap. In collaboration with Dr. Robert Post, a structured interview has been devised to elicit presence of symptoms related to perception, thought processes, orientation, memory, and involuntary motor behavior. The interview is based on a survey of the literature describing such changes in epilepsy. Design of the project involves administering the interview to 120 patients; 40 are patients with a history of major affective illness currently being treated by units of the Biological Psychiatry Branch; 40 are patients with a firm diagnosis of complex partial seizures, under the care of the Epilepsy Branch, NINCDS (in collaboration with Dr. Roger Porter); and forty are patients of the Hypertension-Endocrine Branch, NIHLB, screened for absence of psychiatric or neurological disease. At the present time, approximately 70 affective and epileptic patients have been interviewed. Reliability studies for the interview are now under way. On the basis of preliminary data, it appears that the two groups are similar in incidence of psychosensory symptomatology; it forms a prominent part of the clinical picture in roughly 25% of both groups. We are now beginning to interview the control group. Data will be analyzed to evaluate the relationship of symptoms to course of illness, drug response, and personality characteristics of the subjects.

2. Perception and recall of emotional and neutral stimuli in depression. It is a common clinical observation that affectively ill patients often seem highly insensitive to their own internal emotional state. It is also well known that depressed subjects perform more poorly than controls in a variety of memory tasks. The purpose of this study is to look systematically at how depressed subjects evaluate the emotional qualities of verbal material, how such evaluations change with changes in clinical status, and how they interact with the subjects' ability to recall the material. Fifteen depressed subjects were given a list of forty words to rate for degree of emotionality

on a zero to seven scale. Half the words were high emotion words, and half low emotion words, as determined by previous studies in normals. Subjects were asked to freely remember the words after they had been rated, and later to pick out the words from a list in which they are intermixed with distractors. Fifteen normal control subjects performed the identical task. In addition, whenever possible, subjects who had changed clinical state were again given the list for re-rating. To date, the collection of data is complete and the results are being analyzed. Normal and depressed subjects rate words similarly as to high or low emotionality, although depressed subjects' ratings of the high emotion words tended to be lower, with greater variance than normals. Depressed subjects remember fewer words, both freely, and on recognition, than normals. However, while in the normals there is no relation between emotionality ratings and memory performance, in the depressed group, the lower the ratings the fewer words were recalled. Furthermore, the depressed group showed evidence of preferential storage and retrieval of high emotion words, whereas normals show only preferential retrieval of these words. In the light of previous memory studies with normal and depressed subjects this data suggests that the depressed group processes stimuli more shallowly than normals, and that sensitivity to emotional qualities of words is an indicator of ability to process deeply in the depressed, but not the normal group. That the two groups classified high and low emotion words similarly suggest that the cognitive differences are not at the attentional level, but at a later stage of processing.

3. Hypothesis testing in depression.

Most cognitive research in depression has focused on memory-related impairments. The present investigation uses a learning paradigm which places relatively light memory demands on the subject, focusing instead on hypothesis formulation and testing. The task, devised by Levine, is a variation on the Wisconsin Card Sort. Subjects are presented with the Levine task, which is then repeated with our own modifications. The task involves a deck of cards with two stimuli on each card. Each stimulus has four attributes (such as color and size); the subjects task is to guess which attribute has been arbitrarily designated as "correct." Subjects are asked to point to the stimulus that they think has the chosen attribute, and are given yes or no feedback on selected cards. The procedure is devised so that each administration can be scored for number of hypotheses formed, number of correct hypotheses, ability of the subject to narrow down his choices as more feedback is given, and degree of hypothesis changing or keeping after positive or negative feedback. In addition, our modification of the procedure allows discrimination between poor performance due to memory deficits, or to subjects' inability to formulate an appropriate strategy. Data collection is currently under way. Preliminary results indicate that depressed subjects are unable to focus on a correct hypothesis as well as normals. That this is not due to inattention is suggested by the fact that they form as many coherent hypotheses as normals. Our current evidence also suggests that the deficit is not primarily one of memory on this task, since forcing subjects to adopt an appropriate strategy (without giving them any memory aids), significantly improves performance. Thus, depressed subjects appear deficient in ability to form useful problem-solving strategies. Such a deficit may relate to clinical phenomena such as depressed patients' overgeneralized thinking, and feelings of being overwhelmed by problems.

4. Luria neuropsychological investigation in depressed, schizophrenic, and Alzheimer's patients.

We are currently surveying depressed, schizophrenic, and Alzheimer's patients for deficits in higher cortical functioning, using the standardized Luria examination. The examination is based on the work of the Russian neuropsychiatrist, A. R. Luria, as standardized by Golden and his associates. The battery is divided into 11 sections based on various higher cortical functions, such as motor, tactile, speech, reading, and abstraction. There are also experimental scales assessing overall level of brain pathology, and localization of pathology by hemisphere and lobe. It is hoped that the battery will provide indications for future investigations of specific functions or anatomic areas in these patient groups. Indications to date suggest left temperoparietal dysfunction in the schizophrenic group, and global deficits, including visual perceptual dysfunction in the Alzheimer's. Data in depression is not yet sufficient to provide reliable indications. We are planning to compare Luria results with presence and type of brain abnormality as shown by C-T scan in all three groups. Intergroup comparisons will also examine possible distinct cognitive profiles in demented and psychiatrically-impaired patient groups.

5. Cognitive and mood response to epinephrine in normal and depressed subjects.

This study has three major objectives. The first is to investigate the relationship between epinephrine-induced peripheral sympathetic arousal and performance on a verbal memory task in normal subjects. Effects on learning will be related both to pulse and blood pressure changes, and to subjective mood and activation status of the subjects, as induced by the drug. The second objective is to compare the effects of adrenalin on physiologic and psychologic factors in normal and depressed subjects. Although schizophrenic subjects have been shown to have blunted physiologic responses to epinephrine, the comparable study in major depression has not been done. It has also been shown that autonomic arousal and cognitive set combine to produce a subjective mood state in normal subjects. Since depressed populations are known to have altered cognitive processes, and appear clinically to show deficits in "emotional proprioception," mood response to adrenalin in this group is of theoretical interest. The third objective of the study is to investigate the ability of adrenalin, a peripherally acting drug, to induce state-dependent learning effects in normal and depressed populations. Drug induced state-dependent learning has been demonstrated for a variety of centrally acting drugs, but never with ones whose action is essentially peripheral. The study will also provide insight into the conditions necessary for the production of a dissociative state, in particular, the relationship between state-dependent phenomena, and physiologic, subjective, and behavioral changes induced in the subjects. All phases of the project involve intravenous infusions of the drug at a carefully controlled rate, with continuous cardiac and blood pressure monitoring. The initial phase of the study, involving normal volunteers only, has recently gotten under way. Early data suggests changes in learning and memory, level of activation, and anxiety level, but not other aspects of mood under the influence of the drug.

6. Lateralized hemispheric function in depression. There are presently two distinct parts to this study. The first involves an extensive review of the literature concerning hemispheric lateralization of mood-related behavior and emotional aspects of cognitive processing. Taken as a whole, the current literature suggests that while the right hemisphere appears predominant in the control of many aspects of emotional behavior and processing, there is also a division of function between the hemispheres, with the right hemisphere dominant for negative affects and the left dominant for positive affects. Evidence for these two types of lateralization comes from a wide variety of experimental paradigms and subject populations, each providing a relatively small number of studies. It is clear from the literature that a great deal of confirmatory work must be done in normal, brain-lesioned, and affectively ill populations before the relationship of laterality to mood state can be understood. The literature review is currently being prepared for publication.

The second part of this study involves lateralized tachistoscopic presentation of visual material to depressed and control subjects. The task involves the subject making "same" and "different" judgments on material that can be processed either linguistically (left hemisphere) or on the basis of form (right hemisphere). Differential performance of the two hemispheres will be compared in the two subject groups. This part of the study is currently in the final stages of technical adjustment, and data collection will be initiated soon.

7. Halstead category test performance in depressed subjects. Dr. Robert Savard of the Clinical Center Social Work Department, has been investigating the performance of depressed subjects and matched controls on the Halstead Category Test, a portion of the Halstead-Reitan neuropsychological battery. Data based on the total test scores indicate that depressed subjects, particularly bipolars, and particularly older subjects, produce error rates comparable to patients with organic brain deficits. In this phase of the investigation, two questions are being examined. The first is whether improved performance of depressed patients in the recovered state is due solely to the state change, or whether they are also able to learn while depressed. Current data indicates that the learning curve for depressed subjects is considerably more shallow than for controls. The second question involves difference between patients and controls on the seven subjects of the Category Test. A significant groups-subtests interaction is present between the two groups. This appears to be due to proportionally worse performance by depressives on those subtests most heavily dependent upon spatial aspects of the stimuli. This finding may be suggestive of right hemisphere processing deficits in this group.

8. Visual and olfactory perceptual changes in depression. This study was initiated in order to investigate whether cognitive impairment found in depression might, in part, be due to deficits at the level of elementary sensory processing. Because of the intimate relationship of the olfactory system to limbic areas of the brain that are involved in regulation of emotions, the sense of smell was chosen for the initial phase of the investigation. Threshold detection level, and psychophysical judgments of intensity and pleasantness were obtained from three groups of subjects: depressed, hypomanic, and recovered patients, temporal lobe epileptics, and normal controls. Data on 27 patients, 15 epileptics and 12 controls, has been collected. There are no differences in detection levels of four odorants, two pleasant and two unpleasant, among the three groups. In their ratings of pleasantness and intensity, patients in all three states performed like normal controls. Epileptics, however, supersensitivity to odor intensity and pleasantness in their ratings. This difference appears to occur at a later stage of evaluation rather than at the primary stage of olfactory sensitivity. To investigate the possibility of subtle changes in the depressed group that might have been missed, we are preparing a psychophysical scaling task for two olfactory and two visual stimuli. On the basis of this task, sensitivity and response biases of normal and depressed subjects can be carefully quantitated and compared.

Publications

Silberman, E.K., and Post, R.M.: The march of symptoms in a psychotic decompensation: Case report and theoretical implications. J. Nerv. Ment. Dis. 168: 104-110, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00501-01 LPP

PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Pharmacologic Studies in Depression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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Clinical Neuropharmacology Branch, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.75	.25

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project covers studies examining behavioral and biological response of depressed patients to pharmacologic agents. Three separate studies are involved. The first is an extension of a previous study examining clinical response of depressed subjects to amphetamine; we are now preparing to look at biological response of these patients. The second study involves mood, noradrenergic, and neuroendocrine changes in manic and depressed subjects in response to intravenous clonidine infusions. The third study looks at changes in mood, memory, sleep patterns, and electrophysiology in depressed patients in response to ACTH and its centrally acting analog, ACTH 4-9.

Project Description

This project covers three pharmacologic studies in depression using different classes of drugs, which interact with different neurotransmitter systems. Amphetamine, a drug with well known activating and mood-altering properties, stimulates release of dopamine and norepinephrine. Clonidine is an antihypertensive agent with central noradrenergic agonist actions. ACTH is a pituitary hormone with with actions on both the adrenal cortex and receptors in the brain itself. Organon 2766 is an analog of ACTH 4-9, a fragment of ACTH known to act on CNS receptors, but not on the adrenal.

In the initial phase of the amphetamine study, 18 patients with unipolar and bipolar depression were given 20 mg intravenous amphetamine. The group response consisted generally of activation and mood elevation, although there was considerable heterogeneity in direction of mood change and degree of emotionality following drug administration. Bipolar depression was significantly related to mood elevation following drug. A report based on this phase of the investigation has been submitted for publication. At the present time we are analyzing blood samples drawn during the procedure to investigate correlations of biological response and mental status change. Changes in MHPG levels before and after amphetamine have been reported to correlate with both amphetamine and tricylic antidepressant response; cortisol and growth hormone levels have also been reported to vary with certain aspects of amphetamine response. The present phase of the study will attempt to corroborate these findings, as well as to relate the reactive subtypes found in the clinical study to biological parameters.

Clonidine is a drug with known noradrenergic agonist actions. A significant portion of these actions is presynaptic. It has been demonstrated in animals, for example, that systemic clonidine infusions decreases firing of the locus coeruleus, a noradrenergic center in the brainstem. This center is involved in the mediation of activation and anxiety levels, and success in treating opiate withdrawal with clonidine is presumably due to the drug's moderating action on its activity. Since there are indications of increased noradrenergic activity in mania and anxious depressions, we felt that the action of clonidine in these conditions would be of interest. The protocol involves intravenous administration of 2 micrograms/kg to depressed and manic patients. Mood and anxiety levels are monitored during the procedure; blood samples are drawn for measurement of noradrenergic metabolism as reflected in MHPG. In addition, in collaboration with Dr. Larry Siever, levels of growth hormone, luteinizing hormone, and prolactin are being measured at intervals throughout the procedure. Initial data indicate a possible anti-manic effect, as well as changes in anxiety level in depressed subjects. Preliminary blood analysis suggests a possible blunting of growth hormone response to the drug, as compared to normal controls.

The ACTH study is the final phase of an investigation initiated by Dr. Victor Reus. The study was set up to investigate the effects in depressed patients of ACTH, and an analog of the ACTH 4-9 fragment, a compound known to be active at sites in the brain (particularly hippocampus), but devoid of adrenocorticotrophic effects. Dependent measure included mood ratings, a verbal and visual learning battery, evoked potential studies, sleep studies, and blood assays of cortisol and norepinephrine. Data collection for the study has now been completed, and most of the results have been analyzed. Patients showed no drug effect on mood or memory performance. ACTH administration was found to disrupt REM sleep, while ACTH 4-9 disrupted slow wave sleep. ACTH and ACTH 4-9 were found to have effects on the attentional response of subjects to lights and tones, as measured by average evoked response of the EEG. While ACTH enhances the attentional component of the AER compared to placebo, ACTH 4-9 diminishes it to the point of eliminating the usual attention-stimulus intensity gradient. It is notable that the most prominent effects of these drugs appeared at the longest times after administration, raising the question for future studies of whether peak effects on mood and memory, if any, would appear in a later time frame than the one currently used. In connection with Dr. Dave Pickar, we are now investigating possible interactions between ACTH and the endogenous opiate systems.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00502-01 LPP

PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Atypicality in Major Depressive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.5	1.0	.5

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To explore the possibility of an important atypicality dimension within the group of primary, endogenous depression, we have contructed a rating scale for atypical depressive illness. Forty-four NIMH patients were rated, all meeting Research Diagnostic Criteria for primary, major depressive illness. Atypicality in this group was characterized by lack of encapsulated episodes, interpersonal difficulties, evidence of narcissistic character disorder, and high anxiety and somatization. Though atypical patients were younger than typical, they were hospitalized significantly more often. Biologically, they had smaller variance in two measures of norepinephrine metabolism, as well as lower levels of platelet MAO. Typicals were significantly more likely to have an antidepressant response to sleep deprivation than atypicals. Thus, atypicality as a dimension within primary, major depression may have important theoretical and clinical implications.

Project Description

The initiation of this project was based on our observation of considerable heterogeneity of clinical presentation within the group of patients meeting Research Diagnostic Criteria for primary, endogenous, major depressive disorder. We reasoned that this clinical diversity may contribute significantly to the biological and prognostic heterogeneity within this group. To investigate the possibility of important sub-types, or of an important atypicality dimension within this group, we have constructed a rating scale for atypical depressive illness. The scale is designed to measure degree and type of divergence from a classical syndrome of discrete episodes of autonomous mood and vegetative symptoms.

Forty-four NIMH patients were rated, all meeting Research Diagnostic Criteria for primary, major depressive disorder. In addition, 91% of these met criteria for endogenous depression as well. There were 21 men and 23 women; 28 patients were bipolar and 16 unipolar. In a preliminary session, global atypicality ratings on a zero to ten scale were derived by consensus of trained raters from the psychiatry, social work, and nursing services. Subsequently, the same group consensually rated patients on each of 24 items, to give a total scale atypicality score. Scale items assessed degree of autonomy of symptoms, vegetative signs, personality disorder, neurotic symptoms, thinking and perceptual disturbances, past course of illness, and premorbid adjustment.

Atypicality ratings derived by the two different methods were highly correlated ($r = .83$, $p < .0001$). Atypicality was characterized by relative lack of encapsulated episodes, disturbed interpersonal relationships, narcissistic entitlement, manipulativeness, and prominent anxiety and somatization. Atypicality scores were not related to severity of depression. Atypical patients were significantly younger than typical patients ($p < .002$). Bipolar I patients were significantly more typical than either bipolar II or unipolar patients ($p < .05$), however, the difference no longer reached significance when age was covaried out. Atypical patients were hospitalized significantly more often ($p < .02$) than typical patients, even though the typicals tended to be faster cyclers than the atypicals. Biologically, the typical group had significantly less variance in measures of MHPG metabolism as reflected in urinary MHPG ($p < .05$) and spinal fluid norepinephrine ($p < .03$). While the atypicals tended to have higher MHPG levels, they had lower platelet MAO ($p < .056$). Typicals were significantly more likely than atypicals to have an antidepressant response to sleep deprivation than atypicals ($p = .048$).

The preliminary data cited above suggest that atypicality may be an important factor within primary, endogenous depression. It is not yet clear whether atypicality is best conceptualized as a subgroup or as a dimension within depression. Because in a research setting we are dealing with a selected population, the interpretation of lower age and more frequent hospitalizations in the younger group is not yet clear.

On the one hand, it may be that depression presents in a more varied and atypical manner when the patient is young, but evolves into the classic syndrome later in the patient's course. On the other hand, it may be that the atypical group is distinct from the typicals, with a greater propensity for regressive episodes and hospitalization. It is notable that within our group, atypicality was represented by features of narcissistic character disorder and disturbed interpersonal relationships, rather than by schizoid trends or thinking disorders. Again, this may be a result of screening that would not apply to the general depressive population. There was an indication that the bipolar I group was highly typical, while the bipolar II and unipolar patients were equal in atypicality. Because age and polarity were confounded in our patient population, it was impossible to reliably test for an association between typicality and polarity.

Biologically, the trend toward higher MHPG and lower variance in norepinephrine metabolism can be placed in context of previous biological studies. Generally speaking, lower MHPG has been associated with more classical, and more severe forms of depression. While low platelet MAO has also been associated with more classical (bipolar) depression, it is also associated with a group of people within the general population who have high levels of personality disorder, particularly sensation-seeking and sociopathy. Thus, within our group, atypical patients tended to have low MAO, while typical patients had either high or low MAO.

While the above data was gathered retrospectively by consensus, plans are now under way to rate subjects as they enter the hospital. Working with a fresh, common data base will allow higher reliability than retrospective consensus ratings. Information gathered in this way will enable us to examine correlates of varying types of atypicality, rather than just total scale scores. In addition, we are collecting data on course of illness and response to treatment after NIH hospitalization.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00671-11 LSES

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Social Origins of Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Clarice W. Radabaugh	Social Science Technician	LSES NIMH

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research examines the relationship between social experience and psychological well-being. It looks at the enduring problems people encounter in their daily lives as workers and family members, at the gain and loss of roles as the life-cycle unfolds, and at sudden, unexpected crises that often confront people. Each of these types of experience is examined in relation to symptoms of emotional distress. Data for the study were collected at two times. In 1972 a sample of 2300 people, representative of the Chicago Urbanized Area were interviewed, and a sub-sample (1200) of this group was reinterviewed in 1976-77. Analyses have been made to identify the events that are particularly likely to result in emotional distress, and to understand how people use psychological and social resources to cope with the problems and vicissitudes of life.

PHS-6040
(Rev. 10-76)

Project Description:

This is a longitudinal study of social stress with two interviews conducted at an interval of about four years. The first wave of interviews was carried out in 1972 with a representative cluster sample of 2300 adults in the urbanized area of Chicago. These interviews sought information about three features of people's lives: 1)the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; 2)the coping strategies people employ in dealing with life-problems; and 3)symptoms of emotional stress, anxiety and depression. Then in 1976-77 a second wave of interviews was conducted among a sub-sample of 1200 people drawn from those originally interviewed. Many of the questions that initially asked about chronic role problems, coping, and psychological distress were repeated, thus providing longitudinal information about stability and change on these dimensions of people's lives. Newly constructed questions sought information about key life-events that occurred since 1972. One type of event is represented in the many changes and role transitions that are normative aspects of the life-cycle. A second type of event is represented in the more unexpected, non-normative disruptions of important roles and activities, such as divorce, serious illness, involuntary unemployment, and other life crises. It is the long-range goal of the research to reconstruct from this information those elements of social experience that importantly affect mental health and to identify those coping behaviors and social supports that serve as barriers to psychological harm.

During the past year the emphasis of our work has shifted from identifying the types of events that arouse distress to an analysis of the processes leading to such effects. We are less concerned now with learning which events are likely to produce deleterious effects than in learning how these effects come about. A commonly accepted interpretation holds that life events create distress by upsetting a normal homeostasis, thus leaving the organism vulnerable to physical and psychological harm until a new equilibrium is established. Our results, however, indicate that the manner in which life events come to adversely affect emotional functioning is both different and more complex.

Specifically, we find that where events are related to emotional distress, such as depression, a large part of the effect is indirect. This statement can be illustrated by the results of a path analysis of involuntary job disruptions and their consequences for depression. The analysis revealed the following steps leading from these undesired events to the elevation of depression: first, job loss has a negative effect on income; next, reduced income produces a heightened economic strain, i.e., an intensified struggle to provide the material necessities of life; reduced income and increased strain, in turn, adversely affect self-concepts--the lowering of self-esteem and the lessening of a sense of mastery; these contingencies then lead to depression.

The longitudinal character of our data, it should be noted, methodologically benefits the path analysis described above. First, it is possible to establish a clear temporal order among many of the variables under analysis; second, we are able to observe how changes in one variable are related to changes in another variable, something that cannot be done in looking at data collected at one point in time. Both of these features of the data more easily permit statements about causation that is typically possible with cross-sectional studies. Moreover, because all our measures have been developed through maximum likelihood confirmatory factor analysis, a technique described in earlier annual reports, they have a high degree of reliability. Both the longitudinal nature of the study and the rigor of our measures permit a greater confidence in the results of our path analysis than would otherwise be the case.

We have begun to examine the role of coping responses and social supports as elements that moderate the processes that link life events and depression. Preliminary analyses indicate that social supports are most useful in blunting the distressful consequences of crises events. If crises events become converted into chronic life problems, however, social networks are less supportive. For example, if one loses one's job, family and friends may provide help and encouragement; but this support may dissipate if the job loss turns into a situation of continuing economic deprivation. Supports are typically not sustained in facing chronic problems. It is at this point that people have to rely more on their own coping repertoires. Thus preliminary evidence indicates that social supports and coping have somewhat specialized functions in buffering the distressful consequences of life circumstances, with the former being especially efficacious in blunting the impact of undesired life events, and the latter in vitiating the effects of more enduring problems.

In the next year we shall continue along these lines of analysis. It is becoming increasingly evident that psychological well-being is regulated by a blending of several conditions, not by any single factor alone. Our task will be to identify further how life events, chronic life-strains, coping and social supports come together in shaping the inner emotional states of people.

Publications:

Pearlin, Leonard I.: The life cycle and life strains. In: Blalock, Hubert M., Jr., (Ed.): Sociological Theory and Research: A Critical Approach. Free Press, New York, 1980. pp. 349-360.

Pearlin, Leonard I. Discontinuities in the Study of Aging. In: Hareven, Tamara K. (Ed.): Aging and the Life Course, New York, Guilford, 1980. (In press)

Pearlin, Leonard I. and Radabaugh, Clarice: Age and Stress: Perspectives and Problems. In: Hamilton I. McCubbin (Ed.): Family Stress, Coping, and Social Supports. New York, Springer, 1980.(In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00672-15 LSES

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Social Psychological Correlates of Occupational Position

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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SECTION

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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the re-interviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children.

PHS-6040
(Rev. 10-76)

Project Description:

The principal goal of this research is to assess the relationships between people's occupational conditions and their psychological functioning. The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study was to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators hope to assess with some precision the magnitudes of the reciprocal effects of occupational conditions and various facets of psychological functioning. The study of wives was designed to ascertain whether occupational conditions affect men and women similarly. (On this, see the 1977-78 report.) These data should also enable the investigators to assess the effects of men's occupational experiences on their wives' psychological functioning and of women's occupational experiences on their husbands' psychological functioning, in each case taking account of the individual's own occupational experiences. The study of the children was designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, the major research efforts have been addressed to seven objectives: (1) Using the longitudinal data for men, analyzing the reciprocal effects of job conditions and psychological functioning. (2) Using the data from both the men and their wives, analyzing the reciprocal effects of job conditions and the intellectuality of people's leisure-time activities. (3) Again using the data from both the men and their wives, analyzing the relationships between housework and psychological functioning. (4) Analyzing a thoroughgoing replication, conducted in Poland, of the U.S. findings on the interrelationship of social stratification, job conditions, and psychological functioning. (5) Carrying out another cross-national replication in Japan. (6) Beginning an assessment of the processes by which the educational process affects children's psychological development. and (7) Beginning an assessment of the effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning.

1. Longitudinal analysis of the reciprocal effects of job conditions and psychological functioning. The intent here is a definitive assessment of whether, and if so, the extent to which, job conditions affect men's psychological functioning and whether, and if so, the extent to which, men's psychological functioning affects their job conditions. These analyses began (see the 1976-77 Annual Report) with an assessment of the reciprocal effects of one pivotal job condition--the substantive complexity of work--and one central facet of psychological functioning--intellectual flexibility. In that phase of the analysis, Melvin Kohn and Carmi Schooler demonstrated that the substantive complexity of work has a decided effect, essentially ongoing and continuous, on intellectual flexibility. Intellectual flexibility has a much more gradual (time-lagged), albeit in time even greater, effect on the substantive complexity of work.

The causal model was then expanded (see the 1977-78 Annual Report) to a more comprehensive assessment of job structure and intellectual flexibility. The substantive complexity of work is the single most important occupational determinant of intellectual flexibility, but several other job conditions also affect and are affected by intellectual flexibility.

The next step in the analysis (see the 1978-79 Annaul Report) was the application of the same causal model to each of a number of other facets of psychological functioning--e.g., authoritarian conservatism, self-esteem, anxiety, and standards of morality. These analyses demonstrate the far-reaching effects of job conditions, not only on intellectual functioning, but also on self-conception and social orientation.

During this past year, Kohn and Schooler have attempted to develop a more general causal model of job conditions and personality, a model that simultaneously assesses several job conditions and several facets of psychological functioning. This has proved immensely difficult, primarily because some aspects of self-conception and social orientation are highly correlated with intellectual flexibility--e.g., authoritarian conservatism is so highly correlated with intellectual flexibility that it is not possible with ordinary statistical procedures to estimate their effects on each other or their independent effects on other variables in the model. The investigators have pursued three distinct approaches to this problem, one unsuccessfully, another partially successfully, and the third at too early a stage of analysis to be certain.

One approach, thus far unsuccessful, has been to develop new mathematical procedures for estimating structural-equation models involving linearly dependent variables. A second approach, which can be counted a partial success in that it successfully deals with part of the problem, has been to develop a model of job structure, intellectual flexibility, and those aspects of self-conception and social orientation that are not so closely tied to intellectual flexibility as to pose mathematical problems in a causal analysis. In a sense, this is a model of job structure, intellectual functioning, and "non-intellective" facets of personality. This model validates what had previously been learned from the simpler models, confirming the reciprocal effects of job conditions and several facets of psychological functioning, thus demonstrating that the effects are essentially independent and additive, not simply repeated illustrations of the same basic phenomenon. It also shows that the effects of the psychological variables on one another are entirely plausible, with two interrelated clusters of intra-psychic effects. The first centers on trustfulness, which increases and is increased by intellectual flexibility, decreases and is decreased by fatalism and anxiety, and increases idea-conformity. The second cluster of intra-psychic effects centers on anxiety, which not only is negatively related to trustfulness, but which also decreases intellectual flexibility and is increased by fatalism, lack of self-confidence, and nonconformity in one′s ideas. There is at least a hint here that there may be two separable dimensions of self-conception and social orientation, one centering on trustfulness, the other on anxiety, with both affecting ideational flexibility.

What makes this dual clustering especially intriguing is its implication that there may be two analytically distinct dimensions to orientation (or, more precisely, to those aspects of orientation measured in this study), which would be both substantively interesting and a potential way out of the linear-dependency problem. Kohn and Schooler hypothesized that one principal dimension of orientation is self-direction/conformity, a self-directed orientation centering around non-authoritarianism, holding to personally responsible moral standards, and being trustful of others. They also hypothesized that a second principal dimension of orientation is psychic distress/well-being, psychic distress being measured principally by anxiety, self-deprecation, and lack of self-confidence.

To test these hypotheses, they did a confirmatory factor analysis, using the several facets of self-conception and social orientation as the indicators for two higher level factors, self-directedness and psychic distress. The actual model confirmed their expectations. Self-directedness is reflected not only in nonauthoritaranism, personally responsible standards of morality, and trust, but also (to a lesser degree) in nonfatalism, not being self-deprecatory, and non-conformity in one′s ideas -- all of which is certainly in accord with their basic theoretical position. Psychic distress is reflected not only in anxiety, self-deprecation, and lack of self-confidence, but also (to a lesser degree) in distrust and non-conformity in one′s ideas -- all of which is certainly face-valid.

The investigators are currently working on a causal model of job conditions, intellectual flexibility, self-directedness, and psychic distress. It is too early in the process to predict whether or not they will be successful.

2. Job conditions and the use of leisure-ime. The purpose of this analysis, being conducted by Karen Miller and Melvin Kohn, is to examine the relationship between people's job conditions and the intellectuality of their leisure-time activities. This past year Miller and Kohn expanded the measurement model of leisure-time intellectuality they had earlier developed, to include the intellectual level of the sports in which people actively engage -- this in addition to the number of hours spent watching television, frequency of attendance at plays, concerts, and museums, number of books read, time spent on hobbies, intellectual level of magazines read, and intellectual level of newspaper reading. Adding sports results in a better representation of intellectuality in the leisure-time activities of people at varying educational levels. The measurement models for men and women are very similar.

Using the expanded measurement model of leisure-time intellectuality in causal analysis generally confirms the conclusions previously drawn about the effects of men's job conditions on their leisure-time intellectuality. In particular, the substantive complexity of work has by far the strongest effect of any of the occupational conditions: the more substantively complex their work, the more intellectually demanding is men's use of leisure-time. The only real difference between the earlier findings and the present findings is that a wider range of job conditions significantly affect leisure-time intellectuality in the new model. Routinization, time pressure, dirtiness of the job, risk of loss of job or business, and hours worked all have relatively small negative effects, while job income and heaviness of the work have relatively small positive effects on leisure-time intellectuality. In the reverse causal direction, leisure-time intellectuality affects only the substantive complexity of one's job. Thus, those who engage in intellectually demanding leisure-time activities mold their jobs to be more substantively complex.

The investigators have not yet completed causal analysis of employed women's job conditions and leisure-time intellectuality. Preliminary multiple-regression analyses do indicate that there may be sex differences in the importance of particular job conditions for leisure-time activity. Position in the organizational structure -- i.e., ownership, hierarchical position, and bureaucratization -- appears to have considerably stronger effects on women's than on men's leisure-time intellectuality, while the substantive complexity of work appears to have stronger effects for men than for women. Causal analysis for women will show whether these sex differences hold under more stringent and precise statistical controls.

3. Housework and psychological functioning. On the hypothesis that the psychological impact of the conditions of work encountered in household work would be similar to those of work conditions encountered in paid employment outside the home, Kohn and Schooler included in the 1974 follow-up survey a set of questions about the nature of the actual work performed by the respondent in taking care of the household. Insofar as possible, these questions exactly parallel those asked about work performed in paid employment. The household-work questions were asked not only of housewives, but also of working women and of men.

With this information, Carmi Schooler, Melvin Kohn, Joanne Miller, and Karen Miller have developed measurement models of the basic conditions of work encountered in housework (see the 1978-79 Annual Report). Having created these indices, the investigators are now examining the relationships between housework and psychological functioning. The initial analyses have been limited to women. In these analyses, each facet of psychological functioning is treated as a dependent variable in a multiple-regression equation in which the independent variables are the "job conditions" of housework and relevant social background characteristics.

These analyses reveal striking relationships between the degree of self-direction exercised in housework -- in particular, its substantive complexity -- and several aspects of psychological functioning. There are also relationships between the pressures encountered in housework -- time pressure, heaviness, and dirtiness, for example -- and psychological functioning. In general, these relationships are similar to those previously found for the conditions of work encountered in paid employment and psychological functioning. Just as for paid employment, women whose housework is self-directed are more likely to be intellectually flexible, to have a positive view of themselves, an open and flexible orientation to others, and to value self-direction for their children. Women working under pressured conditions show signs of psychological distress such as anxiety, self-deprecation and ideational rigidity and are likely to value conformity rather than self-direction for their children. However, because of the possibility of reciprocal effects between housework and psychological functioning (e.g., intellectual flexibility may not only be affected by, but may also affect, the substantive complexity of housework), these multiple regression analyses provide only presumptive evidence that housework actually affects psychological functioning.

Causal models depicting the reciprocal effects of housework and psychological functioning are being estimated through the use of structural-equation modeling. These analyses -- not yet completed, but far enough along to permit cautious conclusions -- provide evidence that housework does affect psychological functioning and that the effects of housework on psychological functioning are generally greater than are those of psychological functioning on housework. These conclusions, though, are highly tentative, for much more analysis remains to be done.

4. The Polish replication. The main purpose of this inquiry is to examine the interrelationship of social stratification, job conditions and psychological functioning in a socialist society. Specifically, the study attempts to test, in a different economic, political, and cultural context, Kohn and Schooler's central finding in their studies in the U.S., that occupational self-direction plays a crucial role in explaining the impact of social class on parental values, orientation toward self and society, and intellectual flexibility.

Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. The 1978-79 Annual Report reviewed their efforts to develop methods that assure cross-national comparability of indices. This year, the main thrust of the work has been on causal analysis, addressed to one of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.?

In general, the investigators find the relationships between social stratificational position and psychological functioning to be remarkably similar in Poland and the United States. For example, the correlations between social stratificational position and intellectual flexibility are 0.75 in Poland and 0.77 in the U.S., with education accounting for the lion's share of those correlations in both countries, but with occupational status and income also having an independent effect. Similarly, social stratificational position correlates 0.48 and 0.51 with parental valuation of self-direction in the two countries. Moreover, the relationships between social stratificational position and social orientation are also remarkably similar in the two countries, the correlations being -0.55 and -0.58 for Poland and the U.S. respectively for authoriatarian-conservatism, 0.43 and 0.42 for standards of morality, 0.40 and 0.39 for trustfulness, and -0.18 and -0.18 for idea-conformity. But, for reasons the investigators do not as yet fully understand, the relationships between social stratificational position and self-conception are somewhat different in the two countries. In particular, in the U.S., higher social position is associated with more self-confidence ($r= 0.22$), but in Poland the opposite is true (the corresponding correlation being -0.12.)

Having established the similarity of the relationships between social stratificational position and intellectual flexibility, parental values, and social orientation for the two countries, the investigators are now pursuing the question, whether occupational conditions play as crucial a role in explaining the relationships in Poland as in the United States.

5. The Japanese replication. During the past year another major replication of the Kohn-Schooler survey was conducted, this one in Japan. The replication was carried out by Atsushi Naoi and Kenichi Tominaga of the Department of Sociology of Tokyo University, in collaboration with Carmi Schooler. The replication involved interviewing a probability sample of more than 800 employed Japanese men, using a questionnaire containing all the questions necessary for indexing the structural imperatives of the job and those aspects of psychological functioning measured in the American and Polish studies. This replication is of particular interest not only for the cross-cultural comparisons it will provide, but also because the structure of industry is rather different in Japan, with a much sharper cleavage between the primary and secondary labor markets than is true in the United States.

Data collection took place during the summer and fall of 1979 and the data were coded for statistical analysis in Japan during the 1979-1980 academic year. It is expected that data analysis will begin in October 1980, when Naoi is scheduled to come to the Laboratory as a Visiting Scientist.

6. Educational experience and children's psychological development. The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis is to examine the processes by which students' educational experiences, particularly the degree of self-direction they are able to exercise in their educational endeavors, affect their psychological functioning. In their analyses of social stratification and psychological functioning, Kohn and Schooler long ago found that education has important independent effects on psychological functioning -- greater than those of occupational position, for example. They did not at that time have data to study the processes by which education exerts these psychological effects. Relevant data were subsequently collected in the 1974 follow-up survey as part of the interview with children of the primary respondents. The interview schedule for these "children" -- now aged 13 to 25 -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions are designed to parallel those previously found to be potent for analyzing occupational experience, focusing on such dimensions of the educational experience as its substantive complexity, how closely it is supervised, time-pressure, and the like. The intent is to see whether the concepts and methods developed for the study of occupational experience can be applied as well, and with similar results, to the study of educational experience. In effect, Kohn and Schooler have developed a theory of occupation as an educational experience. Now Miller, Kohn, and Schooler are attempting to apply the same concepts to schooling itself.

The project is in a preliminary stage of conceptualization, data-file creation, and exploratory analysis. Thus far, the investigators have developed measurement models of one basic concept, "closeness of supervision" by the teacher. The models prove valid for students at all educational levels, from junior high school through college. Now in process is an attempt to create measurement models for the substantive complexity of schoolwork, models which may have to be more specifically tailored to particular educational levels. Still ahead is the major task of developing several other measurement models of schooling and of children's psychological functioning. Only then can a systematic causal analysis of educational process and psychological functioning be undertaken.

7. The effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning. Carmi Schooler, Melvin Kohn, and Ronald Schoenberg have barely begun what is undoubtedly one of the most complex analyses of the entire occupations study. The intent is to determine the extent to which, and the processes by which, a married person's job experiences affect his spouse's values, self-conception, social orientation, and intellectual functioning, taking into account the spouse's own occupational experiences and social characteristics. There is some evidence (from Pearlin and Kohn's analyses of the Turin study some years ago) that a man's job experiences affect his wife's values, but the evidence is limited and based on rather primitive statistical methods. The present investigators intend a much more comprehensive analysis, not only of the effects of men's job conditions on their wives' values, but also of each spouse's job conditions on many facets of his partner's psychological functioning, using complex causal models. The inquiry has not yet gotten much beyond testing the applicability of measurement models developed in other parts of the occupations study to the problem at hand.

Publications:

Kohn, M. L.: Job Complexity and Adult Personality. Pp. 193-210 in Neil J. Smelser and Erik H. Erikson (Eds.): Themes of Work and Love in Adulthood. Cambridge, Ma.: Harvard University Press, 1980.

Kohn, M. L.: Personality, Occupation, and Social Stratification: A Frame of Reference. In Donald J. Treiman and Robert V. Robinson (Eds.): Research in Social Stratification and Mobility. New York: JAI Press, Vol. 1, 1980 (In press).

Kohn, M. L., and Schooler, C.: Job Conditions and Intellectual Flexibility: A Longitudinal Assessment of their Reciprocal Effects. In David J. Jackson and Edgar F. Borgatta (Eds.): Factor Analysis and Measurement in Sociological Research: A Multi-Dimensional Perspective. London, Sage Publications (In press).

Kohn, M. L.: Personlichkeit, Beruf und Soziale Schichtung. (With an Introduction by Kurt Luscher). Stuttgart, W. Germany: Klett-Cotta (In press).

Kohn, M. L., and Schoenbach, C.: Social Stratification and Parental Values: An International Assessment. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)

Miller, K. A., and Kohn, M. L.: The Reciprocal Effects of Job Conditions and the Intellectuality of Leisure-Time Activity. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)

Miller, J.: Individual and Occupational Determinants of Job Satisfaction: A Focus on Gender Differences. Sociology of Work and Occupations. (In press)

Miller, J.: Hospital response to the legalization of abortion in New York State: An analysis of program innovation. J. Health & Social Behavior, 20 (December), 1979:363-375.

Schooler, C.: Psychological and Social Perspectives on Status Attainment. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00674-10 LSES

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Social, Psychological, and Biological Interactions in Schizophrenics and Normals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.20	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This line of research has taken two directions this year. One, is the continued use of confirmatory factor analysis to explore the nature of psychophysiological arousal during task performance and to examine the the personality and behavioral correlates of such performance. The second is the analysis of previously collected data in an attempt to discover the theoretical implications of the failure of an apparently well implimented social treatment program for chronic schizophrenics.

PHS-6040
(Rev. 10-76)

Project Description:

Last year saw the development through confirmatory factor analysis of a model of psychophysiological functioning during a perceptual experiment. This year saw an elaboration of the original model and the development of separate models of psychophysiological arousal for both sexes and for both task and rest periods. Initial analyses have also been performed linking such models to individual psychological reactions and behaviors both within and outside of the experimental situation. The first very preliminary results suggest that, although the particulars are somewhat different for each sex, within the sample of college students tested psychophysiological arousal is linked to both good task performance and psychological adjustment.

This year an investigation was also begun of the possible deleterious effects of socially induced arousal on chronic schizophrenics. This work involves the use of linear structural equations analysis to investigate the results of a carefully planned, controlled and measured socially oriented ward treatment program in a Veterans' Administration hospital. The initial analyses have revealed that during the course of the experiment the psychological condition of the patients in the experimental ward became worse relative to those in the control ward. Analyses are now being directed at examining whether this finding may be the result of differential reactions to social stimulation among relatively healthy and sick patients in the experimental and control wards.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00851-16 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Brain Mechanisms of Species-Typical Behavior in Squirrel Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	T.K. Bussard	Biologist	LBEB NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	0.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This long-term project is concerned with identifying brain mechanisms involved in species-typical, prosematic (rudimentary signaling) communication. The mirror display (which is a highly predictable variation of a naturally occurring display used by gothic-type squirrel monkeys) provides a means of testing the effects of brain lesions on various elements of the display. Previous experiments revealed that the medial pallidal segment of the striatal complex, as well as the projections of this segment to the thalamus and to the tegmentum, play a basic role in the display. Tegmental lesions were often complicated by damage to the red nucleus, which is the origin of three evolutionary ancient descending systems. In the present project we are attempting to find out whether or not the red nucleus is implicated in the display.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The striatal complex constitutes one of the three main evolutionary formations of the forebrain. It consists of the olfactostriatum, corpus striatum, globus pallidus, and satellite collections of gray matter. The functions of the striatal complex are largely unknown. Previous work on the present project revealed that the medial segment of the globus pallidus, together with its projections to certain parts of the thalamus and tegmentum, plays an essential role in the species-typical display behavior of squirrel monkeys. In the immediately preceding experiments showing that the tegmental connections are more essential for the display than the thalamic projections, some of the tegmental lesions resulted in damage to the red nucleus. In the continuing analysis, an attempt is being made to learn whether or not the red nucleus is implicated in the display. This nucleus is the origin of three evolutionary ancient descending systems.

Methods Employed: Systematic testing of the mirror display of gothic-type monkeys is employed for investigating the behavioral effects of electrocoagulation of structures of the brain stem (see M-NP-LI-17, 1964). The mirror display is a highly predictable variation of a naturally occurring display used by squirrel monkeys in the show of aggression, in courtship, and in greeting. It consists of five main behavioral components, the incidence of which is scored in two daily tests. After an animal reaches criterion of 80% or more displays in thirty or more trials, the targeted brainstem structures are destroyed by electrocoagulation, and the monkey is tested for several months.

Major Findings: To date the most extensive destruction of the red nucleus has been obtained in squirrel monkey N-5. Volumetric measurements obtained by planimetry revealed that 75% of the magnocellular part of the red nucleus was destroyed in this animal. In the control brain, this part of the nucleus occupies a volume of about 2 mm^3. The lesion in N-5 led to a virtual elimination of the display. The lesion, however, was complicated by bilateral destruction of a major part of the central tegmental fasciculus, which, as shown in last year's report appears to be implicated in the expression of the full display.

Year-long observations on two additional animals (O-5 and P-5) are of control value for the present experiments insofar as they revealed that lesions of the substantia nigra just ventrolateral to the red nucleus and a partial elimination of the rostral prerubral field do not interfere with the display.

Significance to Biomedical Research and the Program of the Institute: In terms of function, the red nucleus is one of the most enigmatic structures of the brain. The same would apply to its anatomical relationship to certain structures such as the striatal complex and the thalamus. The present project is concerned with the question of whether or not the red nucleus is implicated along with the striatal complex in species-typical communication.

Proposed Course: It is technically difficult to electrocoagulate the red nucleus without also damaging the central tegmental tract.(Complicating damage of cerebellar fibers of passage appears not to interfere with the display.) If destruction cannot be limited to the nucleus, an attempt will be made to eliminate it by a chemical substance that will destroy nerve cells, but not fibers.

Publications:

Book: MacLean, P.D., The Triune Brain, Plenum Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00787-01 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Brain Mechanisms of Species-Typical Behavior in Squirrel Monkey: The Isolation Peep

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman, Physiologist	LDN NICHD
	P.D. MacLean, Chief, LBEB	LBEB NIMH
Other:	R.E. Gelhard, Biologist	LBEB NIMH
	T.K. Bussard, Biologist	LBEB NIMH
	C.R. Harbaugh, Biol Lab Tech	LBEB NIMH

COOPERATING UNITS (if any)

J.D. Newman, LDN, NICHD

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the evolution from reptiles to mammals the origination of nursing and parental care were two of the most outstanding developments. Vocalization and hearing became of prime importance for parent-offspring communication. The present project is concerned with brain mechanisms accounting for the isolation peep. Lesions within the central tegmental fasiculus eliminate the production of the peep; lateral tegmental lesions result in infantile types of peeps; while damage to the periventricular and central gray alter the individual quality of the peep.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A cardinal development in the evolution from reptiles to mammals was the development of parent-offspring relationships. Vocalization and hearing became of prime importance in parent-offspring communication. The mammal-like reptiles (the antecedents of mammals) probably had a poor sense of hearing and, like most present-day lizards, may have been mute. Pari passu with the evolution of mammals, the small bones in the jaw joint of reptiles became part of the inner ear, a development that reflects a refinement in hearing. Virtually every existing species of mammals has its own characteristic species-typical vocalization for signaling separation or isolation. Each of two subspecies of squirrel monkeys (the so-called gothic- and roman-type monkeys) has its own typical isolation peep that is readily distinguished by the sonogram. The purpose of the present project is to identify the obviously evolutionary ancient parts of the forebrain implicated in the isolation peep of squirrel monkeys.

Methods Employed: Pre- and postoperative sonograms of the isolation peeps of gothic-type squirrel monkeys are obtained while they are isolated under various conditions from other animals with which they are usually housed in the animal room. In the current phase of the study observations are made on the same animals used in the mirror display study (Z01 MH 00851-17) and which are being subjected to bilateral coagulations of different structures within the thalamic and midbrain tegmentum.

Major Findings: The results may be described under two headings:

1. Elimination of the isolation peep. Two animals failed to produce peeps during a postoperative follow-up of several months. The lesion common to both animals was a bilateral coagulation of the central tegmental fasciculus. One monkey (M-5) in addition had partial bilateral lesions of the pedunculopontine tegmental nucleus, while the other (N-5) had destruction of two-thirds of the magnacellular part of the red nucleus.

2. Alterations in the production of the isolation peep. Four monkeys showed individual alterations in the production of isolation peeps. One monkey (I-5) with an extensive bilateral destruction of the supranigral tegmentum, including ventricular gray matter, appeared as though it were attempting to make isolation peeps, but there was only a "breathy" explusion of air. A second animal (H-5) with a less extensive lesion of the subthalamic tegmentum, together with partial destruction of the periventricular gray, produced abnormal isolation peeps characterized by several harmonic bands below the fundamental frequency. A third monkey (P-5) with a lesion of the periaquaductal gray matter between the third and fourth nuclei produced only rare, short peeps. Finally, a fourth monkey (E-5) with far lateral tegmental lesions in the peripeduncular region, together with partial destruction of the subthalamic nuclei, produced an assortment of isolation peeps that were infantile in character.

Significance to Biomedical Research and the Program of the Institute: There is currently considerable interest in the evolution of vocalization and speech. The interest is timely, because with the present-day threat of extinction of many non-human primate species, there would seem to be, as many would claim, a real urgency to move ahead in this area. The present project involving squirrel monkeys contributes to the understanding of brain mechanisms accounting for the most basic form of vocal communication in mammals generally.

Proposed Course. To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00788-01 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Role of Paleostriatum in Species-Typical Behavior of Chickens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.A. Hagan	Staff Fellow	LBEB NIMH
	P.D. MacLean	Chief	LBEB NIMH
Others:	R.E. Gelhard	Biologist	LBEB NIMH
	T.K. Bussard	Biologist	LBEB NIMH
	C.R. Harbaugh	Biol Lab Tech	LBEB NIMH

COOPERATING UNITS (if any)

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Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: 0.925	PROFESSIONAL: 0.6	OTHER: 0.35

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous comparative studies in this Laboratory have shown that the striatal complex (a basic part of the forebrain in reptiles, birds, and mammals) plays an important role in species-typical behavior. Thus far neurobehavioral studies have been limited to reptiles and mammals. With the initiation of the present project we are extending our observations to birds, focusing on the role of the paleostriatum in the communicative displays and other species-typical behavior of chickens (Gallus gallus). After perfecting an intracleidoic surgical procedure, observations will be made on chickens devoid of all the forebrain except the paleostriatum.

Project No. Z01 MH 00788-01 LBEB

Project Description:

Objectives: The striatal complex constitutes a basic part of the forebrain in reptiles, birds, and mammals. In both reptiles and birds, the striatal complex includes the olfactostriatum (olfactory tubercle and N. accumbens) and the paleostriatum located beneath the dorsal medullary lamina. The present project is part of a comparative investigation attempting to identify the functions of the striatal complex, focusing particularly on its alleged role in species-typical behavior of the chicken.

Methods Employed: In the present phase, we are attempting to learn what forms of behavior remain in the chicken (Gallus gallus) devoid of all the forebrain except the paleostriatum. We are currently trying to perfect intracleidoic surgical techniques for eliminating forebrain structures overlying the paleostriatum (including destruction by aspiration, electrocoagulation, and chemicals). Observations will be made on: 1) growth and early development of behavior; 2) displays; 3) maintenance behavior; and 4) reproductive behavior, including egg-laying. (See the behavioral profile for the chicken, Z01 MH 00789-01.) Time-lapse videotaping will be used for 24-hour behavioral records. After completion of the behavioral observations, the brains of the operated animals will be subjected to both gross and microscopic study. Histochemical and other relevant techniques will be used in experiments concerned with the role of iron, monoamines, and other substances in striatal function.

Major Findings: We are currently perfecting our surgical techniques. To date, two chicks, surgically manipulated on days 19-20 of incubation, have survived; their early behavioral development is being studied.

Significance to Biomedical Research and the Program of the Institute: Other projects in our Laboratory are concerned with analyzing the role of the striatal complex in species-typical behavior of reptiles and mammals. With the initiation of the present project and the extension of our comparative studies to avian forms we hope to obtain further insights into the functions of the striatal complex.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00789-01 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

An Ethogram for the Domestic Chicken (Gallus gallus)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.A. Hagan	Staff Fellow	LBEB NIMH
	R.E. Gelhard	Biologist	LBEB NIMH
	P.D. MacLean	Chief	LBEB NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS: 0.85	PROFESSIONAL: 0.6	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In preparation for a neurobehavioral investigation of the functions of the striatal complex, an ethogram for the chicken is being compiled through a literature search and by direct observations. Time-lapse videotapes and a computer-assisted event recorder are used for the quantification of behaviors used in displays and daily routines.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Our proposed neurobehavioral investigation of the avian striatal complex (see Z01 MH 00788-01) will involve experiments on chickens. For this work, an ethogram for chickens is a prerequisite. An ethogram represents a detailed behavioral profile of a particular animal species. The purpose of present project is to obtain an ethogram of the white Leghorn chicken (Gallus gallus), one of the most readily available domestic breeds.

Methods Employed: The ethogram is being compiled from a literature search and observations of control animals living under semi-natural conditions. Time-lapse videotaped records of the animals are used for analysis of routines filmed over a 3-day period. With the aid of a computer-assisted event recorder, the animals' behaviors can be quantified in terms of the sequence of events and the amount of time spent in various activities.

Major Findings: Chickens' communicative displays have counterparts to those of reptiles and mammals, comprising "signature," challenge, courtship, and submissive displays. The existing literature contains detailed ethological information on the Burmese Red Junglefowl, ancestor of the domestic chicken; and many corresponding observations have been made on the white Leghorn chicken. Further details, however, are required concerning female aggressive behavior, certain hen-chick interactions, flock maintenance, dominant male behaviors, and sequences of daily routines.

Significance to Biomedical Research and the Program of the Institute: In an extension of a comparative study, a detailed behavioral profile of the white Leghorn chicken is necessary for investigating the role of the striatal complex in species-typical behavior of an avian form (see Z01 MH 00788-01).

Proposed Course: To be continued.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00790-01 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

An Improved Hematoxylin Stain for Myelin Sheath

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.K. Bussard	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.0	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With the shortage of hematoxylin in 1973, it was necessary to find a substitute for the standard brand that would serve as a routine stain for myelinated fibers of the brain. A hematoxylin was found that requires no ageing, and with a modification of the Weil-Weigert stain, results in a better staining of the large and fine myelinated fibers than the standard brand.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In 1973, it became impossible to procure the standard form of hematoxylin routinely used in the Weil-Weigert stain for myelinated fibers of the brain. The purpose of the present project was to find another brand of hematoxylin that would serve as an adequate substitute and to make the necessary modifications in the classical method. In experimenting with several forms, one product (Matheson, Coleman, and Bell, catalog # HX0025) was found that did not require ageing and resulted in a finer stain than the standard brand.

Methods Employed: The methodology is part of the major findings described below.

Major Findings: The present hematoxylin method can be used with plain frozen sections or with tissue embedded in gelatin, celloidin, or paraffin. It incorporates an acetone, ammonia, acetate buffer-ethanol sequence that results in wrinkle-free mounted sections and in an enhancement of the quality of the stain. The staining procedure involves defatting, hydration, staining in a hematoxylin-ferric ammonium sulfate solution, differentiation in a dilute ferric ammonium sulfate solution then a potassium ferricyanide-sodium borate solution, dehydration, clearing, and coverslipping. The method has the practical value that it uses a brand of hematoxylin that requires no ageing and reliably stains reptilian, avian, and mammalian tissue. In addition to producing an intense, vibrant stain of large myelinated fibers, the method represents an improvement over classical stains by giving better delineation of very fine fibers. The results are not only consistent, but also aesthetically pleasing, with the large myelinated fibers having a velvety, blue-black appearance, the finer fibers a greyish-black color, and the background a clear tan.

Significance to Biomedical Research and the Program of the Institute: In 1973 when the standard brand of hematoxylin became unavailable, it was necessary to find a substitute for the routine staining of myelinated fibers of the brain. Experimentation with several forms of hematoxylin has resulted in finding one that, with special modifications, results in a better stain than the standard brand.

Proposed Course: To be completed with the publication of the results.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00870-04 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Species-Typical Behavior of Hamsters Deprived from Birth of Neocortex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was not continued into FY 1980. It was completed with the FY 1979 report Z01 MH 00870-03 LBEB.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00874-04 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Neuroethology of Reproductive Behavior: The Role of Endogenous Opiates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was not continued into FY 1980. It was completed with the FY 1979 report Z01 MH 00874-04 LBEB.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00839-03 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

CNS Structures in Mammal-Like Reptiles. I. Forebrain and Epiphyseal Organization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Senior Staff Fellow	LBEB NIMH
	E.C. Roth	Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

Dr. Nicholas Hotton, III, Dept. of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.9	.8	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The early evolution of the mammalian forebrain can be best examined by comparing the progressive changes occurring in brain morphology among ancestral mammal-like reptiles (therapsids). Endocranial brain casts are currently uncommon due to the lack of adequately prepared fossil skulls, their unavailability, and the generally held belief that the walls of the endocranium are poorly ossified. The purpose of this project is to prepare skulls acquired at the Smithsonian Institution for new brain casts. Initial preparations indicate that ventrally the endocranium is partially cartilaginous between the basisphenoid and sphenethmoid bones; however, the preserved endocranium is complete enough to provide valuable information about the size, shape, structure, and encephalization of the brain of mammal-like reptiles. In addition, it is clear that both carnivorous (cynodont) and herbivorous (dicynodont) therapsids will have to be compared for an evaluation of the impact of life style (solitary vs. gregarious groups) upon brain morphology. Current preparations suggest that the brain of dicynodonts is well within the range of reptilian encephalization. The pineal was prominent and contiguous with a well-developed third eye which rested in a cone-shaped parietal foramen posterior to the parietal-frontal suture.

Project Description:

Objectives: Although mammals are undoubtedly descendants of mammal-like reptiles (therapsids), only certain groups of the latter (cynodont lineage) are sufficiently mammalian in terms of skeletal structure to be considered as ancestors of modern mammals. Because gross brain morphology can be correlated with the evolution of animal adaptations, the evolution of the forebrain during the reptilian-mammalian transition should generally reflect the evolution and acquisition of certain categories of adaptation and behavior. Good endocranial casts of the forebrain of mammal-like reptiles do not exist, and only a few hindbrain casts have been reported in the literature. Accordingly, the purpose of the present project is to prepare new endocranial casts and examine the conformation of the forebrain in contiguous lineages of mammal-like reptiles ancestral to modern mammals. Both carnivorous (therapsid) and herbivorous (anomodont) lineages will be compared to evaluate the impact of differing life styles in related groups upon gross brain morphology.

Methods Employed: Fossil skulls with natural fracture lines are being prepared by separating the component pieces of skull and matrix along the fracture lines, removing the matrix with fine preparation tools or acid baths, and reassembling the skull without matrix. Some well preserved cranial vaults of certain specimens selected for their advanced evolution toward mammals have been loaned for preparation by Nicholas Hotton, III, Research Curator, Department of Paleobiology, Smithsonian Institution. With some fossils, parasagittal sections can be made of each skull; the endocranial matrix is then removed from the brain space by use of a pneumatic air hammer and a fine drill equipped with carbide burrs. Once these skulls are prepared, a casting medium (i.e., liquid latex, silicon, or polysulphide rubber) is applied to the endocranial space, allowed to harden, removed, and cured. The endocranial cast is examined for the presence, location, size, and relationship of major brain structures.

Major Findings: Work is proceeding on the brain space of the following mammal-like reptiles: (1) a small cynodont (Sesamodon), #61/53 from the Lower Triassic, Cynognathus zone, Cape Province, South Africa; (2) a partial skull of a therocephalian, #61/107 from the Upper Permian, Cistecephalus zone; (3) a small therocephalian (cf. Ictidosuchops), #64/156 from the Upper Permian, Cistecephalus zone, Cape Province, South Africa; and (4) three dicynodonts, Diictodon sp., from the Upper Permian of South Africa.

In line with previously reported findings on other forms of therapsids, examination of the present brain casts indicates that the cerebrum of cynodonts and dicynodonts was small, long, and narrow and that the olfactory bulbs were large. The parietal foramen in dicynodonts is considerably larger than in cynodonts and housed a well-developed third eye located near the top of the cone-shaped foramen. The presence of the pineal body and its morphological relationship to the third eye is also indicated by intracranial sculpturing in the epithalamic region of the endocranium. Encephalization of dicynodonts appears to be well within the reptilian range and does not demonstrate the enlargement of the forebrain characteristic of Cretaceous or Recent mammals.

Significance to Biomedical Research and the Program of the Institute: Examination of endocranial brain space casts among mammal-like reptiles (225 mya) should yield significant information leading to a more thorough understanding of the evolution of the mammalian brain. Pineal bodies and third eyes are also prevalent in early mammal-like reptiles, but the third or parietal eye was lost as these reptiles acquired a mammalian habitus. The loss of the parietal eye, but retention of the pineal body, may have been a cogent evolutionary event in species adaptations to changing climatic trends. Endocranial casts reflect the temporal acquisition of mammalian brain structure as advanced therapsid reptiles became more mammalian. For example, olfaction plays an important role in mammalian reproduction and communication. The olfactory bulbs in cynodonts are especially large in comparison to living reptiles, suggesting that mammalian olfactory communication was well developed among mammal-like reptiles. In contrast, the cerebral hemispheres are not large in dicynodonts or cynodonts and are still reptilian in gross morphology. Existing endocranial casts indicate that the brains of certain mammal-like reptiles show some morphological (and perhaps functional) similarities with the brains of living mammals.

Proposed Course: To be continued.

Publications: Roth, J.J. and E.C. Roth. The Parietal-Pineal Complex Among Paleovertebrates: Evidence for Temperature Regulation. In Thomas, R.D.K. and Olson, E.C. (Eds.): A Cold Look at the Warm-Blooded Dinosaurs, AAAS Selected Symposium. Boulder, West View Press, 1980, pp. 189-231.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00840-03 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Ontogeny of the Epiphyseal Complex in Lizards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.C. Roth	Staff Fellow	LBEB NIMH
	J.J. Roth	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project was to obtain a record of the developmental stages in lizard neuroanatomy to be used as a guide for future surgical manipulations. Histological materials from this project have been incorporated into ongoing project Z01 MH 00842-03 LBEB, Comparative Neuroanatomy of the Parietal-Pineal Complex in Lizards. Hatchling lizards from this project have been incorporated into ongoing project Z01 MH 00841-03 LBEB, Physiological and Ethological Role of the Epiphyseal Complex in Ectothermic Vertebrates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00841-03 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Physiological and Ethological Role of the Epiphyseal Complex in Ectothermic Vertebrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Senior Staff Fellow	LBEB NIMH
	E.C. Roth	Staff Fellow	LBEB NIMH
Other:	E.S. Nef	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.9	.6	.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In ectotherms, temperature is important as an environmental cue and as a rate-limiting factor in controlling all aspects of reptilian life processes. The parietal eye and pineal body (E-2 epiphyseal system) participate in the control and selection of optimal environmental temperatures and, thus, secondarily affect reptilian physiology and behavior. This project examines the functional relationship between the pineal, body temperatures which permit the expression of species-typical behavior, physiological aspects of reproduction, and the presence of the pineal hormone melatonin. Our finding of melatonin in alligators suggests that the production of melatonin is not limited to the pineal gland since alligators naturally lack pineal bodies.

Project Description:

Objectives: Earlier experiments showed that the epiphyseal complex is intimately involved in the control of temperature selection in certain lizards. Since the activities of ectothermic vertebrates are dependent upon external heat sources for energy, temperature control is a major factor that permeates all facets of reptilian physiology and behavior, i.e., metabolism, reproduction, neuroendocrine interactions, and all social interactions involved in reptilian life cycles. One of the hormones (melatonin) produced by the pineal gland has been shown to be an agent that affects temperature selection and hence, reproduction and metabolism. For examining the role of the epiphyseal complex in reptilian life cycles, one may utilize surgical manipulations to interrupt the influence of all or part of the complex. As surgically epiphysectomized animals mature, (1) physiological and (2) ethological alterations of the normal life cycle will be compared with control animals. Virtually all modern vertebrates possess some portion of the parietal-pineal complex except crocodilians, anteaters (edentates), and dugongs (marine mammals). If pineal bodies and the pineal hormone melatonin are integral components of neuroanatomical systems affecting temperature regulation, physiology, and reproduction, it is important to ask how some vertebrates can function without the pineal or its hormones. Reptilian blood plasma will be sampled for the pineal hormone melatonin.

Methods Employed: In adult animals, surgical extirpation of a pineal body is accomplished through a cranial aperture created by a small dental burr (1 mm). In juvenile lizards, a natural fontanelle surrounding the third eye permits ready access to epiphyseal structures.

1. Physiology - Body temperatures are being recorded by two 24-channel Honeywell multipotentiometers, and reproduction is being examined by specific assays for female lizards (number and size of oocytes, presence of corpora lutea, stage of reproductive cycle, and endocrine condition). Plasma samples from various species of reptiles will be analyzed for the presence of the pineal hormone melatonin by using the techniques of radioimmunoassay (RIA), mass spectrophotometry, and high pressure liquid chromatography (HPLC).

2. Ethology - Ethological data are gathered and condensed into ethograms for each species and for age groups within each species. Experimental effects of pineal extirpation are verified by comparing the ethograms of intact lizards with those of experimental animals. Body temperature data and behavioral inventories are correlated so as to learn the body temperatures at which species-typical displays are most successful in a conspecific communication [e.g., does the dominant animal remain dominant because the challenge displays are performed at or near the most optimal temperature for the information exchange involved in the display?]. Also, the body temperatures of adult animals are compared with behavioral display frequencies (typical intensity) to determine the temperature ranges within which species-typical behaviors are most effective.

Major Findings: 1. Physiology - Pinealectomy results in behavioral selection of warmer temperatures (hyperthermy) than those selected by non-pinealectomized animals. This is the first demonstration of the thermal effects of pinealectomy in reptiles, although a similar effect occurs as a result of parietalectomy. It is not surprising that hyperthermy is a result of these surgical procedures since the pineal body and parietal eye are derived from the same embryological analage. It would be surprising if, in all reptiles, hyperthermy were a common response to parietalectomy or pinealectomy since the variable distribution of third eyes and pineal bodies among reptiles may lead to differences in function among species.

Serum from juvenile alligators was assayed for melatonin. This procedure was carried out because (i) the alligator is a member of the Class Reptilia that does not possess an epiphyseal complex; (ii) alligators are nocturnal and active when titres of pineal melatonin (a potential activity suppressant) are high in animals with pineals; and (iii) the Order Crocodilia is the last modern representative of the large subclass Archosauria to which dinosaurs belong. Although pineal bodies are absent in alligators, pineal melatonin (unrelated to a circadian rhythm) was found in the serum (125 picamoles/ml serum).

2. Ethology - Ethological comparison of epiphysectomized animals begins upon hatching.

A sequence of naturally occurring behaviors has been observed in a group of lizards hatched in the laboratory. These are now being compared to ethograms generated for wild-captured hatchling, juvenile, and adult parietal-eyed and -eyeless species of lizards. Pilot experiments indicate that dominant animals in a population of the lizard Sceloporus undulatus are active at temperatures near the mean eccritic (picked-out) temperature for that species. The body temperatures of subordinate animals are more variable and further from the mean eccritic than those of dominant animals. Parietalectomy (which results in behavioral hyperthermia) tends to result in an increase in the social rank of subordinate animals, perhaps by making the thermal behavior of subordinate animals less variable.

A videotape analysis of the social structure of juvenile lizards has revealed that they are not randomly spaced in an enclosure, but are either touching or close together during more than 90% of their activity period. This finding of affiliation is a significant, although previously unrecognized, aspect of reptilian social structure in the lizard Cnemidophorus velox.

Significance to Biomedical Research and the Program of the Institute: Information about the effects of the epiphyseal complex on temperature regulation, behavior, and reproduction are essential for a better understanding of the ultimate role of striatal and limbic structures in communicative behaviors in reptiles and mammals. Evolutionarily, the stability of the pineal hormone melatonin among all vertebrate classes can be contrasted with the loss of the pineal gland proper. The gland may be expendable but the pineal hormones are indispensable. Alligators apparently create melatonin from extra-pineal sources (harderian gland) that produce chronic low level amounts in each 24-hour (circadian) cycle. Further analysis will determine whether or not (1) melatonin in the alligator is derived from the harderian glands; (2) levels of melatonin are circadian with longer dark periods in the photocycle; and (3) the lunar cycle affects the levels of melatonin at night. These results may be used to examine the evolution of the pineal-less condition (E-0) in some vertebrates.

Proposed Course: To be continued.

Publications: Roth, J.J., Gern, W.A., Roth, E.C., Ralph, C.L. and Jacobson, E.: Non-pineal melatonin in the alligator, *Alligator mississippiensis*. Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00842-03 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparative Neuroanatomy of the Parietal-Pineal Complex in Lizards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.C. Roth	Staff Fellow	LBEB NIMH
	J.J. Roth	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.6	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Current information on the gross and microscopic anatomy of the parietal-pineal complex among lizards is scarce and incomplete. This project examines the anatomy of pineal systems and their neural connections with the forebrain in a comparative survey including parietal-eyed and -eyeless forms. Analysis indicates that some embryos of parietal-eyeless lizards also lack lateral eyes. Gross anatomical characteristics of the pineal body vary from what has been described in other published reports in the degree of development and amount of physical contact with the parietal eye. Existing variances in the literature derive from sampling techniques which have not reflected the normal histology or morphology of the majority of animals in any given species. Early references indicate that members of the reptilian order Crocodilia possess pineal glands. Subsequent reports were unable to demonstrate pineal tissues in these reptiles. We have demonstrated (Z01 MH 00841-03 LBEB) the presence of the pineal hormone melatonin in alligators which are being examined to attempt to clarify the question of presence of the pineal.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Both the human pineal gland and other mammalian pineals are often presented as representing the vertebrate pineal in both morphology and physiology. Actually, the mammalian pineal is but one part of a complex of neural structures, including a third eye, that find their most complete development among subavian vertebrates, especially lizards. This project examines the morphological and neuroanatomical range of epiphyseal systems among reptiles. Since secretory pineal glands comparable to those of mammals and birds have evolved among certain reptiles, a survey of pineal systems among reptiles can be used to compare and derive models for the evolution of mammalian and avian pineal systems and to expand the information for reptilian epiphyseal systems. The course of development of the pineal and parietal eye is being examined in developing embryos of parietal-eyed and parietal-eyeless lizards. This information will be used to examine the neuroanatomy and histochemistry of the epiphyseal system.

The allometric relationship between the brain and its endocranial space is being examined in lizards and crocodilians as they mature. Determination of age in reptiles is very difficult since they exhibit continuous growth. One predictable measure of reptilian age, not considered before, is the size and relative state of ossification of the parietal foramen, the cranial housing of the third eye.

Methods Employed:

Neuroanatomical - The brains of lizards representing parietal-eyed and -eyeless species are being examined histologically with routine paraffin methods to determine the amount of variability to be expected from individual to individual. Afferent connections of the fully developed parietal eye and pineal body are being examined using the horseradish peroxidase technique. A modified gelatin embedding technique and Weil stain has been developed for this work. Members of the reptilian order Crocodilia which do not possess pineal bodies but which do have the hormone melatonin (principally secreted by the pineal gland in other vertebrates, see Z01 MH 00840-03 LBEB) are being examined for the presence of vestigial pineal neural tracts.

Techniques used to visualize neural fibers in mammalian and avian material present difficulties in use with reptilian brains. A modified Bodian stain has been developed to visualize reptilian fine fibers. A modified iron stain is used to demonstrate the extent of pineal tissue in reptiles since in other vertebrate classes, the pineal area stains heavily.

Morphological - In the past, the brains of reptiles have been estimated conservatively to occupy 50% of the endocranial space. In order to acquire a more realistic estimate of brain to endocranial space ratios, we are comparing the relationship between the brain and the endocranial space in lizards and alligators as they mature. This information is acquired by measuring fresh brain weight and volume and comparing these data with those from endocranial casts. The resultant ratios (brain volume/endocranial volume x 100) will be used to modify existing encephalization quotients using the Jerrison formula $EQ_i = E_i/E_e$ where EQ = encephalization quotient, E_i = actual brain size, and E_e = expected brain size.

Parietal foramen morphology in an age sequence of the lizard Anolis carolinensis will be assessed from prepared skulls. Allometric changes in the skull will be used to subtract growth changes occurring in the skull and foramen as a result of an increase in size from those changes that occur as a result of foramen ontogeny.

Major Findings: The survey of the epiphyseal system of the lizard Anolis carolinensis indicates that the pineal body connects to the parietal eye in all animals examined. These data differ from published reports indicating that the pineal and parietal eye of Anolis carolinensis are anatomically separate structures. Histological examination of the pineal complex in the lizards Sceloporus undulatus, Sceloporus graciosus, and Uta stansburiana shows the same anatomical connections observed in Anolis carolinensis.

Horseradish peroxidase (HRP) injections into the parietal eye of Anolis carolinensis led to precipitation in the pineal body and the area of the habenula and posterior commissure. Further observations on the transport of HRP molecules from the parietal eye to deep brain structures may help to clarify the function of the epiphyseal complex of these ectothermic vertebrates.

In Alligator mississippiensis and Cnemidophorus tigris septentrionalis, a staining technique that has demonstrated high concentrations of iron in specific areas of the brain of birds and mammals (including the pineal) showed little if any iron present in these reptiles. Spectrophotometric iron analysis is planned for selected areas of the reptilian brain.

The histology of unhatched lizard embryos lacking fully developed lateral eyes exhibits abnormally developed pineal bodies. A functional or trophic relationship between the ontogeny of the visual system and the photoresponsive pineal gland may exist.

Preliminary results indicate that the brain fills approximately 85% or more of the endocranial space in young alligators and 70% through 80% in young lizards. These data differ from reports in the literature that conservatively estimate the relationship as 50%. The brain of sexually mature alligators occupies less of the endocranial space (60%) than does the brain of younger animals. These new values should provide a more realistic basis for estimating encephalization quotients in living and fossil vertebrates.

Although not noted in the literature, ossification of the parietal foramen (foramen closure) follows a predictable sequence in animals of increasing size (snout-vent length) and presumably age. The ontogeny of ossification of the parietal foramen in Anolis carolinensis begins as an "H" shaped fontanelle, passes through a triangular shaped stage, and eventually assumes an elliptical configuration or closes completely. Among fossil skulls, available growth stages of Daptocephalus (a mammal-like reptile) indicate that some parietal foramen closure occurred as maturity was reached in this species. A species of stem reptile, Captorhinus aguti, also shows closure of the parietal foramen as individuals become larger; however, the pattern is different from lizards in that the H-shaped fontanelle does not exist in our youngest specimens, and foramina assume an elliptical configuration during all of the growth stages that have been examined.

Significance to Biomedical Research and the Program of the Institute: The different phases of this project are important in revealing the evolution of mammalian pineal systems and are fundamental for establishing a framework for assessing normal pineal morphology and histology in modern reptiles. Our extensive survey of epiphyseal systems will help to generate a classification of reptilian epiphyseal systems that may provide a new understanding of the evolution of pineal structures.

The neuroanatomical connection of the pineal gland and parietal eye, the deposition of histochemical tracers from the parietal eye in the region of the pineal gland, habenula, and posterior commissure, and the relative lack of iron (centrally linked to aerobic metabolism) in the brains of reptiles may help clarify the function of the epiphyseal system in thermoregulation in ectothermic vertebrates that employ a highly anaerobic metabolic mode.

The findings on hatchling lizards naturally lacking lateral eyes and possessing abnormal pineal bodies suggest a functional or trophic relationship between the ontogeny of the visual system and the photoresponsive pineal gland.

Endocranial casts are becoming increasingly useful in understanding the evolution of the brain since they indicate the encephalization levels of living and fossil vertebrates as well as the involvement of various sensory systems in species evolution. Our studies indicate that endocranial casts may be far more informative for some species than is currently assumed since casts of the endocranial space can closely approximate brain structures. The ossification and closure pattern of the parietal foramen may be helpful in determination of age in living parietal-eyed forms and may also be applicable to the study of age structure in fossil parietal-eyed forms. These findings can be applied to modern and fossil vertebrates, thus helping to identify similarities in brain morphology between ancestral mammal-like reptiles (see Z01 MH 00839-03 LBEB) and their descendants, the mammals.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00875-03 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Respiration-related Units in Forebrain of Squirrel Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.H. Orr	Guest Worker	LBEB NIMH
	P.D. MacLean	Chief, LBEB	LBEB NIMH

COOPERATING UNITS (if any)
R.H. Creecy, DCS, ADAMHA
M.J. Eckardt, ADP, ADAMHA
W.J. Vaughn, RS, NIMH

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

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NIMH, ADMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In a preceding project, the entrainment of respiration by vagal volleys revealed that units in certain parts of the forebrain discharged in phase with respiration. Earlier work on the present project demonstrated that units in several parts of the forebrain discharge spontaneously in phase with respiration. Because of the variation in duration of the respiratory cycle, an analysis of the data presented special statistical difficulties. In the present phase of the work a satisfactory computer-assisted statistical analysis has been achieved for the continuing work on respiration-related units.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In preceding studies (see Z01 MH 00860-03) on awake sitting squirrel monkeys, the entrainment of respiration by vagal volleys revealed that some units in certain striatal and limbic structures discharge in phase with respiration. In the present experiments, we are attempting to learn the incidence of spontaneously occurring respiration-related units in the forebrain. The beginning of inspiration is used to trigger a recording of unit activity. Because of the variation in the duration of the respiratory cycle, an analysis of the data presents special statistical difficulties. The current phase of the project is concerned with resolving the statistical problem.

Methods Employed: A computer is used to detect the times of occurrence of each spike relative to the peak of inspiration, and both the relative and standardized spike times are calculated. The one-sample Kolmogorov-Smirnov (K-S) test is used to test the null hypothesis. A phase independent modification of the K-S test (Kuiper's test) is applied when the distribution is expected to be cyclic. A graphical output includes event triggered histograms for both relative and standardized spike times, together with their smoothed versions obtained by the Parzen window technique.

Major Findings: As just described, a satisfactory computer-assisted statistical analysis has been achieved to meet the special requirements of the present study concerned with the detection of respiration-related units in the forebrain. This represents the major finding of the present phase of the project.

Significance to Biomedical Research and the Program of the Institute: In the preceding project, the entrainment of respiration by vagal volleys revealed that some units in certain forebrain structures discharge in phase with respiration. In the present project it has been shown that units in several parts of the forebrain discharge spontaneously in phase with respiration. The significance of these discoveries has yet to be demonstrated. It would be of great consequence if it were shown that the discharges of respiration-related units affect central excitatory states that underlie wakefulness and attention and other psychological functions of the forebrain.

Proposed Course: A next step in this research is to investigate the effects of sleep on respiratory-type neurons in the forebrain.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00867-02 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Ascending Vagal Projections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief	LBEB NIMH
	T.K. Bussard	Biologist	LBEB NIMH
	A.H. Orr	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.2	OTHER: 0.6

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In preceding microelectrode studies the entrainment of respiration by vagal volleys revealed the presence of units in the putamen and basal limbic structures that discharged in phase with respiration. The present neuroanatomical project employing autoradiography and retrograde labeling of neurons with horseradish peroxidase has provided evidence that the parabrachial nucleus (part of the so-called pneumotaxic center) may transmit respiratory-related nerve impulses to some of the forebrain structures in question.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In previous projects it was shown in squirrel monkeys that coincident with the entrainment of respiration by vagal volleys, about 6% of units in the putamen and certain basal limbic structures discharge in phase with respiration. In a follow-up study using the peak of inspiration to trigger the recording of respiratory cycle, it was found that units in these same structures discharged spontaneously in phase with respiration. The purpose of the present project is to learn whether or not there are ascending projections from the solitary nucleus to the pneumotaxic center of the pons and thence to the forebrain that could account for the respiration-related discharges in question.

Methods Employed: Autoradiographic sections were prepared from the brains of three squirrel monkeys in which 0.1 μl of (2, 3, -^{3}H)-proline was injected under direct vision into the solitary nucleus (suboccipital approach). For retrograde labeling of neurons with horseradish peroxidase (HRP) the putamen and amygdala were the sites of injections in three monkeys.

Major Findings: In one of the preparations there was a remarkably well-defined localization of the autoradiographic marker in the solitary nucleus at a level just rostral to the obex. Dense labeling could be traced forward in the solitary tract as well as in a number of laterally directed fibers. The most rostral extent of the labeling was in the lateral parabrachial nucleus on the same side at a level just caudal to the decussation of the fourth nerve. In dark fields the labeled nerve terminals appeared as a bright cuff bordering the lateral part of the brachium of the superior cerebellar peduncle.

Significantly, injection of HRP into the amygdala resulted in a retrograde heavy labeling of cells in the lateral parabrachial nucleus that was at the same level and corresponded in distribution to the autoradiographic labeling just described. Accordingly, the combined findings provide neuroanatomical evidence of a bisynaptic pathway leading from the solitary nucleus to the parabrachial nucleus and from there to the region of the amygdala. Among significant ancillary findings were the labeling of neurons in (1) the supratrochler nucleus that corresponds to the dorsal raphe nucleus of the rat, (2) the peripeduncular nucleus and, (3) the thalamic periventricular nucleus.

Injections of HRP into the putamen resulted in labeling of nerve cells in the paracentral, central lateral, and medioventral part of the centre median nucleus, as well as in the compact part of the substantia nigra.

Significance to Biomedical Research and the Program of the Institute: The mechanisms by which information of visceral origin may influence functions of the forebrain are largely unknown. Answers pertaining to this question are requisite for progress in understanding psychological functions of the forebrain. The integration by the forebrain of information from the internal and external environment is not only necessary for answering to somatovisceral needs; it is also believed to be essential for such psychological functions as memory, conditional learning, emotional experience, and a sense of personal identity. Moreover, the flow of nerve impulses from the internal environment to the forebrain are involved in gradations of awareness and of sleep. The present findings of ascending connections from the solitary nucleus to the parabrachial nucleus of the midbrain and from there to certain parts of the forebrain add significant comparative details to the recent accumulation of observations pertaining to this matter.

Proposed Course: To be concluded with the publication of the results.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00786-01 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effect of Hyperbaric Oxygen on the Pallidum, Substantia Nigra, and other Iron-Rich Structures of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

Capt. Nicodemus, Hyperbaric Research Laboratory, Naval Medical Research Institute, Bethesda, MD

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies in other laboratories have shown that specific areas of rat brain are damaged following repeated exposures to oxygen at high pressure (OHP). The map of OHP damaged areas closely matches our map of iron-rich areas of the brain. Using the cupric-silver method to detect neuronal degeneration, we examined animals which had been exposed daily to OHP for 1-5 days to determine the earliest appearance of degeneration in the various brain areas. Areas most severely affected after 1 or 2 exposures were the auditory nuclei. These nuclei contain relatively moderate amounts of iron but have a high metabolic rate. Other areas with high metabolic rate but with no iron displayed no damage under OHP. Areas with the highest amount of iron--globus pallidus and substantia nigra--were barely affected. It is concluded at this stage of the study that damage to areas of the brain is a function of both iron content and rate of metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Multiple exposures of rats to oxygen at high pressure (OHP) have been shown to cause complete necrosis of globus pallidus and substantia nigra as well as other areas. Since pallidum and substantia nigra are focal points of damage in Huntington's chorea and Parkinson's disease, respectively, experiments to induce damage less than complete necrosis in these areas using OHP was deemed valuable.

Methods Employed: Male and female Sprague-Dawley rats were exposed daily for 1-5 days for 50-60 min to 100% O_2 at 57 psi (OHP). These conditions duplicate those of earlier studies by Balentine. A special "decompression" sequence was employed to minimize pulmonary congestion which usually resulted in death by anoxia due to irritation of the lungs. The decompression was not necessary under these conditions to prevent the "bends." While allowing a fairly rapid decompression to atmospheric pressure within 5-10 min, the sequence maintained the chamber at 100% O_2 for 20 min and then a gradual admission of atmosphere mixture during the next 20 min. Oxygen levels were monitored with a Beckman chromatograph and CO_2 levels, by an infrared spectrophotometer.

Two days after the last exposure, all animals were anesthetized and perfused with an aldehyde solution in preparation for the cupric-silver method.

Major Findings: Brain sections treated with the cupric-silver procedure displayed degeneration of neuron cell bodies as well as dendrites, axons, and synaptic boutons. Severe degeneration was present even in animals exposed a single time to OHP. Distinct brain areas affected to varying degrees are listed in order of severity. HEAVY: dorsal-caudal limit of entorhinal cortex and the auditory structures (superior olive, trapezoid body, cochlear nucleus, lateral lemniscus, inferior colliculus core, and medial geniculate). MODERATE: nucleus of the facial nerve and interpeduncular nucleus. SLIGHT: substantia nigra, mediodorsal thalamus, lateral habenula, central amygdala, and globus pallidus. Repeated exposures increased the degree of degeneration seen, but changes between exposures were not extreme.

Adjacent brain sections stained for Nissl substance with thionine show that severe loss of neuron cell bodies is not evident even though the silver sections displayed extensive degeneration after 1-2 exposures.

The areas displaying degeneration are the same areas which exhibit iron when treated by the Perl's method for ferric iron. The only exception is the dorso-caudal strip of entorhinal cortex which shows no iron.

A comparison of maps of iron distribution and degeneration due to OHP with maps of metabolic activity (obtained with 2-deoxyglucose) shows that neither high iron nor high metabolic rate alone is sufficient to mark an area as susceptible to damage by OHP. Rather, areas with moderate amounts of iron and of high metabolic rate are damaged by OHP.

Significance to Biomedical Research and the Program of the Institute: Globus pallidus and substantia nigra are two areas of the brain which contain high concentrations of iron and also are involved in at least two of the most perplexing neurodegenerative diseases: Huntington's chorea and Parkinson's disease. The cause of the atrophy and death of neurons associated with these diseases is not understood. Since pallidum and nigra contain high amounts of iron and iron is a well known initiator of membrane-damaging free radicals when allowed to interact with molecular oxygen, an error in the processes which segregate iron and O_2 into respective metabolic compartments could cause eventual cell death. This part of the study confirms that a breakdown in these compartments through exposure to OHP can cause precipitous cell death.

The location of iron in the cytoplasm of glia and neuropil and not in the cell bodies of neurons which undergo degeneration in these areas after OHP suggests an important interaction between neurons and glia.

The current experimental approach did not allow a selective degeneration of the high iron pallidum or nigra alone, but other manipulations might allow such selection. Another factor must be sought which, when coupled with high amounts of iron, can cause a gradual demise of the neuron populations within the striato-pallidum and nigra.

Proposed Course: To be concluded with the publication of the results.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00844-02 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Detection of Proteins in Two-Dimensional Polyacrylamide Electrophoretic Gels: A Highly Sensitive Silver Stain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

Dr. Carl R. Merril, Laboratory of General and Comparative Biochemistry, NIMH

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SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The use of a silver staining protocol provides a highly sensitive means of visualizing individual proteins of a complex mixture that have been separated by 2-dimensional electrophoresis. The procedure for silver staining described earlier (Z01 MH 00844-01 LBEB) uses a chemical development of a latent silver image to yield a visual display. Creating an image by physical development, however, uses seven-fold less silver, detects proteins with comparable sensitivity, is technically easier, and yields gels with lighter backgrounds.

Project Description:

Objectives: In the past, physical development of latent silver images provided a well controlled and sensitive means of development, especially for astronomers. In the present project, we are using physical development of silver nuclei bound to proteins in 2-dimensional polyacrylamide gels to produce a clearer background and a more controlled reaction than the procedure used previously (Z01 MH 00844-01 LBEB, 1979).

Methods Employed: Two-dimensional gels were treated as before (see Z01 MH 00844-01 LBEB) up to and including incubation of the gels in a cupric and silver nitrate solution. Instead of proceeding into a diammine solution with subsequent reduction, the gel is taken through the following sequence:

1. 1% citric acid - 10 min
2. 0.5% acetic acid - 5 min
3. Physical developer - 20-30 min

The physical developer consists of three parts which are mixed together just before use.

Part A: 5% sodium carbonate
Part B: 0.2% Ammonium nitrate
0.2% Silver nitrate
1.0% Tungstosilicic acid
1000 ml distilled H_2O
Part C: same as Part B plus 7 ml of 37% formaldehyde solution

The development is stopped by a bath of 0.5% acetic acid.

Major Findings: Our use of physical development to register the silver laden proteins in electrophoretic gels has several benefits: 1) it requires seven-fold less silver; 2) it is technically easier; 3) it has comparable sensitivity to our earlier silver method; 4) it is less susceptible to the staining of gel surface artifacts; and 5) it permits better control of the image development. Improved control of development allows a clearer background which enhances the contrast of the protein spots or bands.

Significance to Biomedical Research and the Program of the Institute: This Silver Stain II procedure offers yet another means using silver to visualize the constituent proteins of a complex mixture contained in an electrophoretic gel. Not only is there improved economy with this method through its lower requirement for silver, but because of its technical simplicity, batches of gels may be processed on a fixed time schedule.

In the course of development of the images of protein spots with both silver methods, a difference in color has been observed which may provide yet another dimension by which these proteins as gene products can be analyzed and compared.

The clearer background obtainable with the Silver Stain II procedure will allow better analysis by computer imaging and comparison of gel patterns.

Proposed Course: To be concluded with the publication of the results.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00845-02 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparative Anatomy and Histochemical Characterization of the Islands of Calleja

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Hill	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Research on this project has been held in abeyance during FY 1980. It is anticipated that research will resume in the first quarter of FY 1981.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00846-02 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Ovarian Hormones on Iron Content of the Pallidum and Substantia Nigra

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Hill	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: .9	PROFESSIONAL: .65	OTHER: .25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The sex difference in hepatic and serum iron, as well as the changes seen in iron metabolism during pregnancy, are attributed to the effects of ovarian hormones on the uptake, mobilization, and storage of iron. We have determined through histochemical means that a sex difference in brain iron exists in the rat and that the levels of iron in the brain appear to change with changing hormone levels whether these fluctuations are naturally occurring or induced. By spectrophotometric means we measured iron levels in the iron-rich globus pallidus and substantia nigra of male and female rats. The effects of naturally fluctuating hormones (as in estrus and pregnancy) on brain iron levels were also determined with this method. Both histochemical and spectrophotometric analysis indicate that a sex difference in brain iron exists, with females having the greater amount, and that brain iron levels vary during estrus and pregnancy.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Earlier studies in this laboratory using histochemical visualization of brain iron suggested differences in levels of brain iron between sexes. Since sex differences had been found for liver iron in rats, measurements of brain iron by spectrophotometric means to detect effects of ovarian hormones were undertaken.

Methods Employed: Samples of the globus pallidus (GP) and substantia nigra (SN) were obtained from 270 µm thick slab of rat brain by micropunch and pooled to form an "iron-rich" brain sample to be compared with iron-poor micropunch from cortex. Iron levels were determined by spectrophotometric means. Iron levels in serum and liver were also determined for each animal.

Three groups of rats were examined: 1) females in normal state of estrus, 2) ovariectomized females, and 3) castrated rats of both sexes which had estrogen implants and injections of progesterone.

Major Findings: The spectrophotometric determination of pallidal and nigral iron levels of female and male rats demonstrates a sex difference, with females having significantly more iron. This sex difference in iron is paralleled in the serum and liver where females also have higher levels of iron. These data are tabulated below (all Student's t-tests df=18). Units are µg of Fe/g wet weight except serum which appears as µg of Fe/ml.

	Female	Male	
GP + SN	9.2 ± 2.81	5.7 ± 1.70	p<.01
Cortex	5.7 ± .50	5.3 ± .91	NS
Liver	139.7 ± 12.35	75.9 ± 8.31	p<.01
Serum	2.8 ± .45	1.8 ± .35	p<.01

In addition, preliminary results with this method indicate that: 1) brain iron levels peak during pregnancy, following the same pattern of change as liver iron and 2) brain iron levels fluctuate during estrous cycles.

Significance to Biomedical Research and the Program of the Institute: Iron deficiency is a prevalent nutritional disorder in human beings. Affected individuals frequently exhibit behavioral symptoms including irritability, fatigue, and (in adolescence) decreased quality of scholastic performance. Little data is available concerning the effects of iron metabolism on brain functions. The results of the present study show that female rats have about 1.5 times more brain iron than males. There is also evidence that brain iron levels fluctuate in those conditions in which ovarian hormones fluctuate, as in pregnancy and during the estrous cycle. It is assumed that the sex difference in iron metabolism is related to the demands of pregnancy and the necessity for a supply of iron for the developing fetus. The sex hormone effect on cerebral iron is possibly implicated in the physiology and behavior of reproduction.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00871-04 LBEB

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparative Histochemical Analysis of the Ventral Striatal Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer	Senior Staff Fellow	LBEB NIMH
Other:	J.M. Hill	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.6	.15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Further evidence for the presence of a ventral component of globus pallidus in the olfactory tubercle was obtained by comparing the characteristic staining patterns of acetylcholinesterase (AChE), succinate dehydrogenase (SDH), and by measurements of the size of neuron cell bodies. In AChE stained sections, some neurons of the dorsal globus pallidus are stained heavily for this enzyme. A similar pattern is found in olfactory tubercle. This supportive evidence is matched by the consistency of the display of neurons stained heavily for the enzyme SDH in both ventral and dorsal pallidum. Preliminary size class analysis of neuron cell bodies reveals that large size classes are heavily represented in ventral pallidum. Other observations suggest that the large size classes of cells tend to be AChE-positive. These data provide further support for the hypothesis of a pallidal component in the tubercle.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A further characterization of (1) striato-pallidal complex by analysis of size classes of neuron perikarya and (2) distribution of perikarya which stain heavily for acetylcholinesterase (AChE) and iron was prompted by observations of nonuniform AChE patterns in brain areas which are iron positive. These observations suggest subdivisions of pallidum not previously appreciated.

Methods Employed: The sizes of neuron perikarya of the globus pallidus, dorsal and ventral compounds, in rat were determined as follows. Sections stained for RNA (Nissl substance) were projected (2000X magnification) and the outlines of cell bodies traced. The areas within the traced outlines of the perikarya were measured with a polar compensating planimeter.

The acetylcholinesterase distribution in rat and squirrel monkey revealed by a modified Koelle method. Through short incubation periods or presacrifice administration of an organophosphate (DFP) which inhibits AChE, neuron perikarya which synthesize AChE could be visualized even in areas where background staining for AChE is normally very dark.

Iron distribution was revealed using Perl's method for ferric iron on formalin fixed brain sections from rat, squirrel monkey, chicken, turkey, and turtle.

Major Findings: In rat, neuron cell bodies of the pallidal formation, including the component extending into olfactory tubercle, tend to be larger in the more ventral zones. The sizes of cells found dorsally are hardly represented in the ventral areas in this limited sample. Further examination of the rat and other lissencephalic mammals is required to verify this gradient of sizes in pallidum.

Subjectively, the cells of largest size appeared to be located medially and somewhat ventral in the dorsal pallidum. This finding was of particular interest since the DFP treated rats showed the highest concentration of AChE-positive cell bodies in this same area.

Sections stained for succinate dehydrogenase (SDH) reveal intensely stained cell bodies in the pallidal complex. These cells seem to be uniformly distributed and of medium size; however, a quantitative study is needed to confirm this. These SDH cells occupy the ventral extension of pallidum below the anterior commissure, thus providing further confirmation of the pallidal character of deep layers of the olfactory tubercle.

Significance to Biomedical Research and the Program of the Institute: Analysis of the rat pallidum by histochemical demonstration of AChE and SDH enzymes and cell size reveals a more elaborate view of pallidum than was previously recognized. The data suggest a partitioning of the main dorsal pallidum into cell populations that have correlates in the primate brain. It is currently believed that the endopeduncular nucleus of rat is the approximate homologue of the internal segment in a primate. The data of this study do not totally support this notion. The AChE picture of rat's endopeduncular nucleus does not match the squirrel monkey's internal pallidum; rather, it compares with a large population of cells in squirrel monkey which are believed to be part of the cell population known as Meynert's nucleus basalis.

Establishing exact homologues between primate brains and experimental animal brains is crucial if the latter are to be used as a basis for predicting situations and conditions found in human brains. Despite all that has been attributed to pallidum and its suggested involvement in human disorders, we know little of its comparative morphology and histochemistry.

Proposed Course: To be concluded with the publication of the results.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00872-03 LBEB

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparative Study of Olfactory Projections to Neopallium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The projection of the olfactory bulb in opossum to the neopallial part of insula cortex was established using the cupric-silver method for detecting degeneration as reported in last year's report Z01 MH 00872-02 LBEB. This study was completed at that time.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00834-04 LBEB

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Social Group Organization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James L. Hill	Visiting Associate	LBEB	NIMH
Other:	John B. Calhoun	Research Psychologist	LBEB	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The spatial and social organization of (1) freely growing and (2) regulated growth laboratory populations of house mice at different stages of crowding are being examined. In freely growing populations, the social structure consisting of small, spatially distinct groups collapsed as the density increased. The whole population merged into a single, homogeneously distributed group as the mice began to lose their individual sociospatial identity. In a population in which the growth rate is regulated, spatially distinct social groups are maintained for longer periods during each generation and, at a higher population density than in freely growing populations.

Project Description:

Objectives: To determine the principles of group organization, structure, and intragroup communication that operate to foster development and maintenance of optimum-sized groups within (1) freely growing and (2) controlled growth populations. The social roles of individuals within groups; the contributions of environmental complexity and resource distribution patterns to group stability; and the contribution of group stability to population survival are being investigated.

Methods Employed: Data analysis for this project is proceeding in three stages; the first is complete, the second is nearly complete, and the third is part of an ongoing study. The study and the methods used are as follows: (1) For data from the freely growing populations, cluster analytic techniques (including varimax rotation) are used to develop factor-loadings or weightings of association scores in the large matrices analyzed. The data analyzed by these methods are the inter-animal distances between diurnal resting site locations by all animals in three freely-growing laboratory populations of house mice. These data are collected during each of fourteen censuses conducted during the early phase of population growth. Animals are assigned to groups on the basis of their degree of association with the mathematical factors identified by these procedures. Various statistical methods have been used to determine stability of these groups in time and space. (2) The degree of association of each individual to the center of its group has been correlated with other aspects of the individual's behavior to determine if central group individuals influence group structure and stability more than peripheral individuals. (3) For the controlled-growth population, data collected both early and late in each generation are being examined to determine how increasing density influences social group formation and stability of each generation. The relationships between group structure and arrangement of environmental resources in all of the populations is also being studied.

Major Findings: Our findings as they relate to the three stages: described above are: (1) In a freely growing population in the early phase of growth the population is organized into several spatially discrete groups which frequently change location and membership. As density increases individuals tend to become spatially fixed, and there is a coalescence of discrete groups into one, large, homogeneously distributed group. This collapse is facilitated in an environment where resources, such as food, water, and nest sites, are distributed in a contiguous, even pattern. (2) The closeness of an individual's relationship to a group is, in part, related to age, sex, and population density. Young animals remain near the core of a group; females are more frequently found at the periphery of a group; the proportion of animals associated with group centers decreases with increasing density. (3) In the early phases of growth in a controlled growth population, groups tend to remain more fixed both socially and spatially than in a freely growing population. The environment of the controlled-growth population is structured so that it is readily divisible into recognizable physical units. This environment plus the slowed rate of population growth may be the factors that help individuals to maintain their sociospatial identity within a particular group even when the population becomes crowded.

Proposed Course: To be discontinued upon termination of the present controlled-growth population study.

Significance to Biomedical Research and the Program of the Institute: Many animals, including human beings, are adapted to develop and live most effectively within small, stable, social groups. However, urban induced anonymity, frequent change of residence, and crowding reduce the probability that meaningful interactions will occur at the appropriate intensity and frequency. Our studies of the principles of group organization among rodents contribute to the understanding of the requirements of group living.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00835-04 LBEB

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Crowding and Social Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Hill	Visiting Associate	LBEB	NIMH
Other:	J. B. Calhoun	Research Psychologist	LBEB	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS: 3.1	PROFESSIONAL: 0.6	OTHER: 2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of the project is to determine the behavioral, ecological and reproductive factors which characterize a population of house mice (Mus musculus) at each of several successive stages of crowding. The rate of population growth has been regulated: Once the adult population reached 100, the population was allowed to double approximately every 200 days. After 1200 days the population reached about 1800 and then began to decline because the death rate exceeded the rate of recruitment. At present, although many pups are born each day, none are reared to the age of weaning. If this lack of effective reproduction continues for another 200-400 days (until the youngest generation becomes reproductively senescent) the population will decline to extinction.

PHS-6040
(Rev. 10-76)

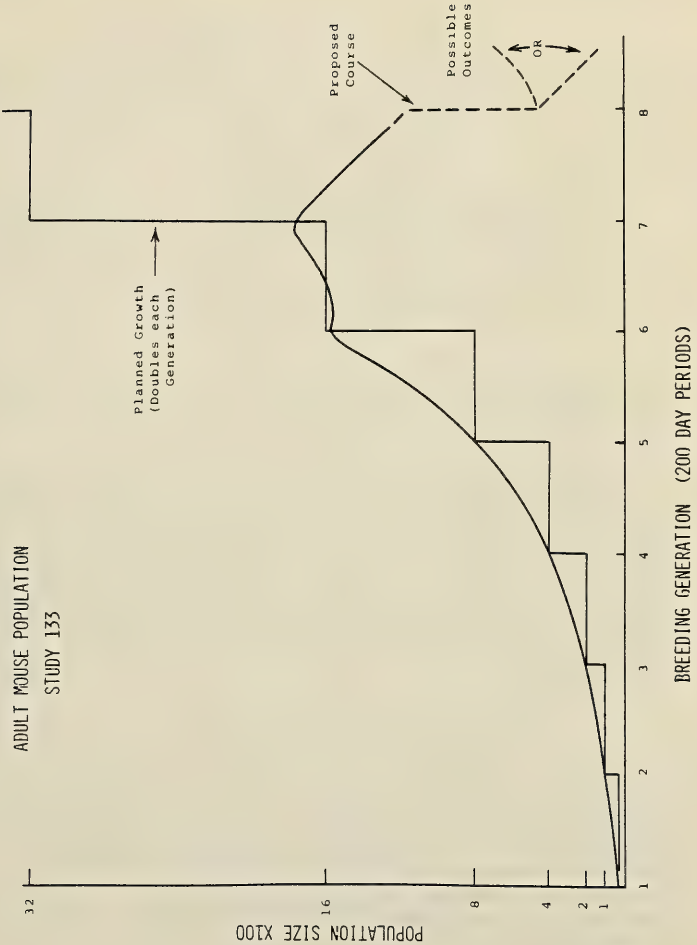
Project Description:

Objectives: To determine how (i) the physical structure of the environment and (ii) the behavioral and social history of members of the population influence composition and stability of social groups in a controlled-growth population as it passes through stages of increase, stability, reproductive senescence and decline.

Methods Employed: All procedures of handling the mice and making observations follow an approximate 200-day recurrent cycle. During the first 140 days of the cycle all progeny born are removed before they in turn produce progeny. This tactic provides adults with experience in rearing young. The final 60 days of each 200-day cycle is devoted to establishing the next generation. Enough young are permitted to survive to double the entire population from the number of adults present. By this strategy, each successive generation is produced by experienced, reproductive aged survivors of the two prior generations. Identification of each mouse is accomplished prior to weaning by the attachment of a pair of numbered metal ear tags. In a predetermined census schedule all mice are captured during the least active phase of their daily activity cycle when these nocturnal animals are associated with their preferred diurnal resting residences. Analysis of this resting residence data can reveal membership and length of association of social groups. Other data collected are related to reproductive performance, the distribution in the habitat of the total population, adult population, juvenile population, and infant mortality.

Major Findings: The growth of the population is represented by the smooth line in the accompanying figure. The break in the ever increasing number of animals about the time of the beginning of the production of the seventh generation was accompanied by the following changes in behavior: 1) in adult males, territorial behavior was being replaced by abnormal nipping behavior; 2) juvenile males spent much of their active time forming and reforming into clusters of animals sitting side-by-side, all facing in the same direction on the low partitions that subdivide the environment; 3) females exhibited an increase in aggressive behavior; and 4) the level of recruitment of young animals into the population was low because of high infant mortality.

The seventh generation breeding period began approximately one year ago. For the first 30 days of the production of the seventh generation the population declined because old animals died and no young survived to weaning age. During the following 200 days, although over 560 young survived to weaning age, the population increased only by about 200 animals because of the high death rate of old animals. During approximately the last 150 days there has been further decrease in population because none of the eighth generation infants has survived beyond three days and the mortality rate of adult animals has remained high. The lack of effective reproduction despite a relatively high pregnancy rate (see table 1), may be occurring, in part, because the animals of reproductive age tend to remain



ADULT MOUSE POPULATION
STUDY 133

so crowded that new-born pups are unavoidably trampled. The crowding continues even though more nest sites become available as the population decreases.

TABLE 1

Present Pregnancy Rate/Generation

Generation	Mean Age	NT	NF	N Pregnant	(%)
7	200+	476	243	78	(32)
6	400+	660	270	27	(10)
5+ older	600+	133	33	1	(3)

If the self-initiated crowding behavior in the population persists until the latest generation becomes reproductively senescent, the population will decline to extinction. The influence of crowding on reproduction has been tested by removing sixth generation bisexual pairs and housing them as isolated pairs or in small social groups; successful reproduction among these pairs approaches 100 percent.

The design of the habitat, which is clearly demarcated into readily recognizable units of physical space with which individuals can associate, has proven to be an effective feature which allowed the population to reach more than double the anticipated optimum size.

Proposed Course: To remove all but the seventh generation animals. This will reduce the reproductive age population to approximately the size of the fourth breeding generation which reproduced at a high rate, and because the pregnancy rate of the seventh generation is relatively high it is anticipated that this population will prove adequate for testing our hypotheses concerning long term effects of crowding. It is expected that if the effect of crowding upon reproduction is acute, then, the decrease in population density should result in the return of successful reproduction. If crowding effects are chronic, then the population should decline to extinction.

Significance to Biomedical Research and the Program of the Institute: In an environment structured to facilitate normal social behavior, the effects of crowding are lessened, and the population may grow to a greater number than otherwise possible. However, the effects of overcrowding eventually do appear: stress, deviant social and parental behaviors, and resultant population decline. Our research on crowding in a rodent population not only points out that the structure of the environment is important to normal social behavior, but also indicates that normal social and parental behaviors can resume if individuals are removed from stressful, overcrowded conditions. These results suggest that the reaction of organisms to stressful social conditions may be ameliorated in environments appropriately structured to facilitate normal social behaviors.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00836-04 LBEB

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Conceptual Adaptation and Evolution

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Calhoun	Research Psychologist		LBEB	NIMH
Other:	R. W. Ridel	Research Psychologist		LBEB	NIMH
	G. A. Bagley	Computer Programmer		LBEB	NIMH
	H. E. Cascio	Electronics Engineer	DRS	BEIB	NIH
	D. R. Ahuja	Research Fellow		SPH	HU
	R. A. Newell	Electronics Engineer		STD	NIMH

COOPERATING UNITS (if any)
None

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TOTAL MANYEARS: 3.4	PROFESSIONAL: 1.2	OTHER: 2.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hybrid rats, Rattus norvegicus, are used to investigate a model of non-genetic adaptation with rats living in a stable environment under crowded conditions. A control population received food and water under normal conditions with no demands for learning cooperative tasks placed upon them. Rats maintained at twice normal population density obtained food and water resources by learning cooperative tasks. Results indicate that these latter rats show (1) reduced intensity of fighting, (2) increasing maintenance of body weight, and (3) development of modes of reproductive control, including killing of young. We conclude that acquisition of cooperative behavior reduces crowding related stress.

PHS-6040
(Rev. 10-76)

Project Description:

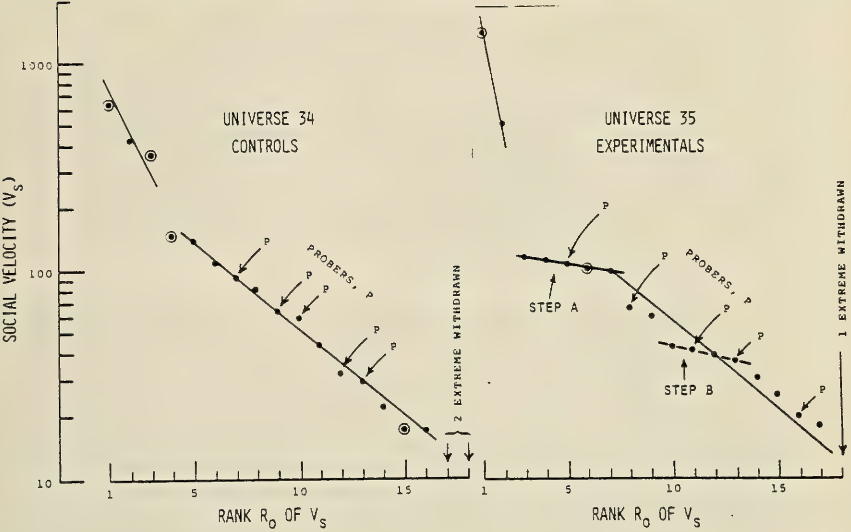
Objectives: To determine the effects of an investigator-induced acquisition of cooperative behaviors by rats living at twice optimum density in contrast to a control population without the opportunity to acquire cooperative behaviors.

Methods Employed: Subjects are Norway rats of an F_2 generation derived from a double F_1 cross of 2 domesticated and 2 wild strains. The F_2 subjects were born in the complex habitats to parents living at optimum density. Experimental F_2 lived from weaning under the demand for cooperative drinking, i.e., one rat must be present in each of 2 adjacent wire tunnels for either one to obtain water by pressing a lever. Control F_2 are maintained on ad lib access to food and water. In addition to their normal sources of food and water, members of both populations from early adulthood have had access to three different reward situations, each with a different reward: saccharine water, sunflower seed, orange quarters. After 3 months of adaptation, during which individual response to each situation provides reward, the apparatuses available to each population will be altered to demand cooperative behavior in order to obtain rewards. A computer data acquisition system nearly continuously monitors movement between habitat compartments and response to food and water acquisition apparatuses by identity of each rat. Each 21 days every rat is examined for reproductive and health status, and 16 hours of recording of visual observation is made.

Major Findings: The COOP experimental subjects, in contrast to the DISOP controls, exhibit: (1) less intense fighting with fewer wounds received; (2) sustained greater body weight; (3) decreased conception; (4) increased killing of recently weaned young by adult females. These results acquire meaning when viewed against a finding of a difference in the distribution of "social power", the ability to engage in, and to exercise dominance during, social encounters. Social velocity, V_s, measures social power. $V_s = (v \times S_I)^{1/2}$, where v, a measure of crude velocity, is taken as the number of passages between habitat compartments, and S_I is a social status index: $S_I = T(D/S)$, where D is the number of times a rat is dominant, and S the number of times subordinate, and $T = D + S$. S_I reflects the number and outcome of status in interactions. Plotting V_s as a function of the rank, R_o, of V_s reveals social system organization just as clearly as measures of anatomy and physiology do for the organization and function of individual animals. Such an analysis for males is shown below.

The 2 or 3 most dominant males in each population exhibit a marked elevation of power. Power is distributed among the remaining controls as predicted by our earlier developed theory: The difference in power or social velocity between any two adjacent ranked (R_o) individuals is a constant. In contrast there are discontinuities or "steps" in the curve for the experimentals. Such steps in the distribution of power indicate a reordering of its distribution among members from one steady state to another, or the settling out of a steady state from a condition of social pathology and uncertainty.

POWER AS SOCIAL VELOCITY (V_S) AMONG MALES



This last statement raises an important point about what is a finding or item of data. The points in the graphs are already second order data derived from "objective" raw data, while the graphs are third order data representing how the social power held by individuals forms social structure. Were an X ray of a femur to reveal irregularities of as gross a nature as that for social power among our experimental rats, one would conclude a prior fracture and insert this information as a datum in the patient's history. Our past theory development, and analysis of other populations, leads us to accept as a datum a prior "fracture" of the social system, not yet healed.

Then we tentatively accept apparent relationships between antecedent conditions and such fourth-order data as suggesting fifth order data, which might be designated as interpretations. For example: We note that experimentals have been subjected to much more environmental uncertainty with reference to the contingencies of water acquisition. Either temporary equipment failure or different ways in which rats learn COOP behavior have made behavior by experimentals much more variable. Systems theory and research indicate that any introduction of increase in system variables produces instability and prolongs reachievement of organizational stability. We thus accept as tentative fifth order data the relationships between environmental uncertainty and changes in behavior by the COOP experimentals such as (1) more rapid learning of the three test apparatuses; (2) reduction in conception; and (3) instability of social organization.

Proposed Course: The completion of this project will culminate a 25-year effort to gain an understanding of how social behavior and information processing within the context of a population system contribute to the understanding of evolution. In the pursuit of this endeavor we have developed many new concepts as well as many new technologies for studying the behavior of rats. These resources will be utilized in a major new direction proposed for initiation by the Unit for Research on Behavioral Systems. We will explore the role of the neocortex in the ability of rats to cope with complexity and uncertainty. Normal control subjects will be compared with neopalliectomized experimentals. Different sized groups of each type will inhabit special environments which vary in size, complexity and behavior-related contingencies.

Significance to Biomedical Research and the Program of the Institute: Humans are exposed to environments which are increasingly becoming more variable, complex, and changing. This historical trend has increased our capacity to create and utilize ideas as well as to cope with change. However, the current rate of change introduces undesirable stress on individuals and produces broader instabilities in society. Our research with rats suggests that promotion of adoption of more types of cooperative social roles can ameliorate stressful consequences of physical and social environmental change. However, introduction of cooperative social roles, even though they have an eventual ameliorative impact, also produces instabilities in social relationships which last longer than does the learning of the new cooperative manners of behaving. This leads to a generalization: Maximal profiting from introduction of new social roles must allow for a relaxation interval of time, prior to introduction of another new cooperative social role.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00837-04 LBEB

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cooperation and Crowding Induced Alterations in Vocalizations by Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James L. Hill	Visiting Associate	LBEB	NIMH
Other:	John B. Calhoun	Research Psychologist	LBEB	NIMH
	W. J. Vaughn	Systems Programmer	STD	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vocal communications among members of free ranging populations of rats housed in complex laboratory enclosures are either audible to humans, or are above the upper limit of human hearing (ultrasonic). Ultrasonic vocalizations tend to be more prolonged and to form patterned sequences. Rats vocalizing simultaneously are capable of utilizing separate frequency bands so that each signal is distinguishable. Rats required to perform cooperatively appear to exhibit different and, possibly more complex patterns of vocalization, than those rats with free access to food and water resources.

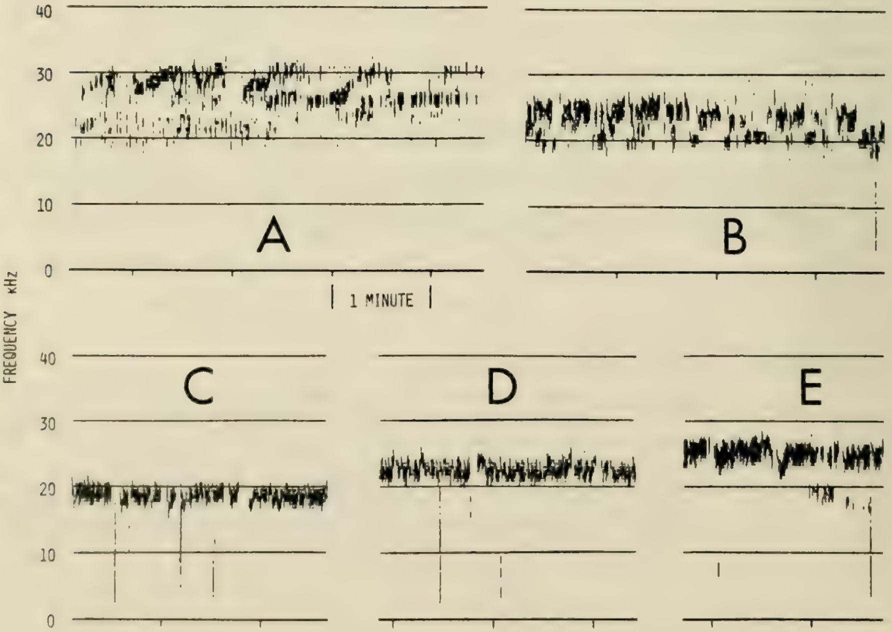
PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine if the quality, quantity and pattern of rat vocalizations change when the animals are required to perform complex cooperative behaviors to meet their physiological needs.

Methods Employed: Vocalizations detected by a B&K condenser microphone (Dynamic range = 20 Hz - 200 kHz) with a parabolic reflector, are classified according to type and frequency by the URBS PDP 11/04 computer coupled to a Tektronix 5L4N Spectrum Analyzer. Graphic output is of two major types: (1) showing the trace of the power curve (see FY79 Project Report); and (2) the other represents each vocalization as a vertical line spanning the frequency band of the sound (see example below under Major Findings). Simultaneous recording of vocalizations, direct observations, and computer assisted determinations of rat activity are being made to determine the types of vocalizations that are associated with specific behaviors. This vocal-ethogram will provide the base line for comparison of experimentally induced changes in vocalization or behavior.

Major Findings The tracings in the figure below are Type 2 graphic representations of the vocalizations of free ranging rats.



Most of the sounds in the four examples above are above the normal range of adult human hearing, and, therefore, are classified as "ultrasonic" vocalizations. The short duration vertical lines between zero and 18 kHz represent audible sounds such as squeaks and sounds resulting from movement of rats through the environment. Sample sections A and B depict what may be the equivalent of "conversations". These vocalizations occur both sequentially and in simultaneous choruses which are evidenced by as many as three simultaneous distinct peaks of sound. C, and D depict the vocalizations of single individuals; the use of different frequency bands in each instance may indicate either that different information is being transmitted or that there are individual differences in the pitch of the voice. In record E, two rats vocalized for about 40 seconds, utilizing clearly distinguishable frequency bands. The individual which was vocalizing at the lower frequency ended its vocalizing with a loud, audible squeal. Note that the vocalization at the higher frequency apparently was not influenced by that at the lower frequency, suggesting that the sounds were related to separate events. All of these examples are short segments excerpted from vocalizations which continued for periods lasting up to 20 minutes and more. At present, we are correlating the occurrence of these and other patterns of vocalizations with visual observations of social behavior and computer records of movement patterns.

Proposed Course: To continue the comparison of the vocalizations of rats required to perform cooperative behaviors with those of rats that can satisfy their needs *ad lib*, and to add a comparison of the vocalizations of neopalliectomized rats to those of normal rats to determine the contribution of the neocortex to the regulation of the type and patterning of vocalizations.

Significance to Biomedical Research and the Program of the Institute: Vocal communication, which plays a major role in mammalian social behavior, may become changed as an outward manifestation of stress or some altered brain function. We present here an animal model that is being used (1) to discover how long term stress influences vocal communiation and (2) to investigate the role of the neocortex in the regulation of vocal communication. Furthermore, the extremely complex and prolonged patterns of vocal communications between rats suggest that Norway rats, *Rattus norvegicus*, may lead to the use of this species as an animal model for the study of the early evolution of communication that culminated in human language.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00838-03 LBEB

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Theoretical Population Dynamics: An Evolutionary Human World Population Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dilip R. Ahuja	Research Fellow	SPH	HU
Other:	John B. Calhoun	Research Psychologist	LBEB	NIMH

COOPERATING UNITS (if any)

None

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SECTION
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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES x☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The last step of an evolutionary human world population model has been completed. Staggered inception of more rapid population by socio-political units dominated by a neighboring unit fully account for the historical trend in which each doubling of world population takes only half the time as the prior doubling.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop a minimum number of assumptions about socio-demographic conditions relating to the growth of human populations that will enable construction of a model of past world population growth conforming to the historical trend of each sequential doubling taking only half the time as that for the prior doubling.

Methods Employed and Major Findings: Methods and findings in the present strategy of model building form a cyclic, recursive, step-wise process in which the results of one cycle of analysis become the data or the guide for employing a different method of inquiry. This procedure may proceed through several cycles before realization of initial objectives.

Method No. 1: Our initial data base consisted of a set of recently published estimates of population size for many subregions of the world that encompassed the past three thousand years. The mathematical characteristics of population growth over time of each subregion was determined.

Finding No. 1: Every subregion exhibited the same process: An exponential rate of growth would continue for several generations, and then abruptly shift to a greater rate of growth, which also lasted for several generations.

Methods Nos. 2 and 3: and Findings Nos. 2 and 3: The above steps were taken on sequentially larger aggregates of subregions. Results were of the same nature as for Step 1.

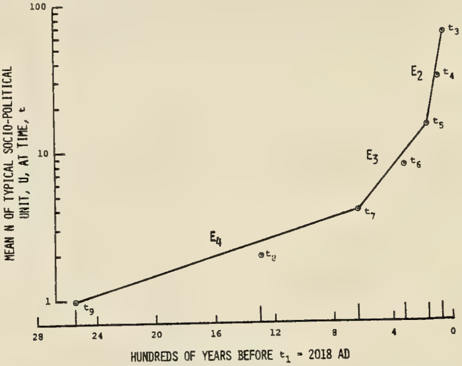
Method No. 4: Results from Steps 1 to 3 were re-examined to find repetitive patterns of population change over time.

Finding No. 4: (1) In any set of contiguous regions or socio-political units, U, the times of initiation of increased growth varied such that the later the initiation of growth, the faster the following growth; (2) the faster the rate of growth, the larger the population of the unit, U, became before a comparable cycle began, and (3) the length of such cycles roughly coincided with duration of time encompassing two doublings of world population.

Method No. 5 and Finding No. 5: Finding No. 3 of Step 4 required clarification of a troubling alternate hypothesis in order that the directions suggested by Step 4 could be followed. If at each second doubling of world population all members of every set of contiguous units, U, increased at the same rate, then, as shown in Figure 1, a family of straight lines would result. Here times t_3 to t_9 are times of attainment of successive doubling of world population, dated backward from 2018 A.D., t_1, a date when capabilities of human reproduction and longevity can no longer sustain the pattern of increase in world population that continued over the past several thousand years. A curve fitting all of times

t_3 to t_9 conforms to the von Foerster curve which characterizes observed world population growth. Therefore, since population size for times t_4, t_6 and t_8 are under estimated in Figure 1, it follows that some precise patterning of staged time delays of increased rate of population growth among neighboring regions must ensue.

Figure 1

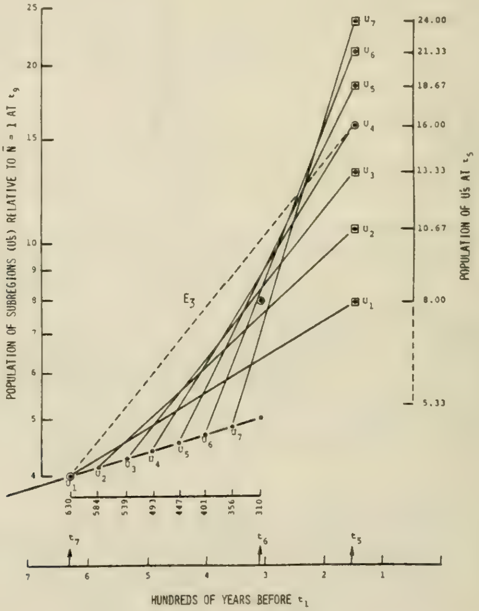


Steps 6 to 9: These involved successive approximations utilizing variations of (1) the base line rate of growth from which increases start, (2) the time to the last region for which growth is initiated, (3) the size of the terminal populations of the regions forming the interacting set. None of these were completely satisfactory, i.e.; they did not produce results exactly conforming to the expectations of the von Foerster equation. However, they eventuated in a satisfactory set of assumptions fulfilled in Step 10.

Method No. 10: and Findings No. 10: Assumptions: (1) Spatial characteristics producing nearest neighboring regions or units, U, demand that there are seven units in each set, and that the central member dominated the others at times of socio-political union. (2) Times of initiation of population growth must all be encompassed between the population doubling at which socio-political amalgamation takes place and the following doubling. (3) The initiated increases in population must continue to the second population doubling following amalgamation of regional units, U. (4) Terminal populations of units, U, vary according to the principle that power among member units is a constant. The terminal population size of a unit is taken as the measure of power. (5) The minimal population size of a regional unit in a set at the completion of a cycle of growth is equal to the average population size at the prior doubling of the average population size of the units in a set. (6) The base line growth rate of units, from which their later increases start, is that resulting in increases in population produced by a continuation of the constant rate of population growth for the past cycle as shown in Figure 1.

These six assumptions culminated in Figure 2, which represents Finding No. 10: This figure utilizes a representative cycle of population increase, that between times t_7 and t_5. Summation, for any representative sample of times between t_7 and t_5, of the populations of the seven regional units, results in total population sizes exactly as predicted by the von Foerster equation which gives change in population size over time such that, from any point in time, each successive doubling in total population takes only half the time as for the prior doubling. These ten recursive steps culminated in realization of our objective.

Figure 2



Significance to Biomedical Research and the Program of the Institute: The character of our recursive analysis and model building strongly suggests that there is one, and only one, solution to a description of how the uniquely human greater than exponential growth of world population has taken place. Since our model must be very close to the true solution it may be taken as a fairly accurate framework for understanding historical cultural change. The model reveals that cyclic major changes produce continuous instability, and such instability must be the major requirement for evolution to continue. This instability during cultural evolution has, according to our overall theory relating to the present project, produced a 32,000-fold increase in negentropy represented by human life. There has been a 1000-fold increase in population and a 32-fold increase in conceptual capacity of the average individual. But as this cultural-historical process now terminates, an evolution-terminating consensus of opinion is emerging. This opinion holds that our goal should be to work toward stabilization of population size and environmental fluctuation. Policy implementation of this opinion will likely foreclose the possibility of further evolution, including any advances we wish to profit the human species.

Publication:

Calhoun, J.B., and Ahuja, D.R.: Population and Environment: An Evolutionary Perspective to Development. In Hauser, P.M. (Ed.): World Population and Development. New York, Syracuse University Press, 1979, pp. 80-98.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00843-02 LBEB

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Conceptual Prosthesis of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Calhoun	Research Psychologist	LBEB	NIMH
Other:	Garrett A. Bagley	Computer Programmer	LBEB	NIMH
	D. Michael Murtha	Guest Worker	LBEB	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our theory of how concepts are associated in the brain holds that 1625 preverbal experiential concepts form a network of relationships. This theory guided development of a concept associational index to 2400 paragraphs from 162 authors. Use of this index permits selection and sequencing of paragraphs across authors to develop creative insight-stimulating manuscripts. This index is also being used to refine the theory of how concepts are associated in the brain.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop a method of indexing that will aid computer selection and reassembly of excerpts from many authors into synthetic manuscripts capable of enhancing our creative insights.

Methods Employed: 10,000 pairs of words which shared meaning were identified from a set of 7,500 paragraphs. These paired associations permitted definition of a 3-dimensional network of 1625 concepts, most likely having preverbal connotation. They radially differentiated into more complex concepts in seven steps from the central concept represented by the word "tradition". Utilizing this theory, a permutated associational index was prepared of different concepts, each associated within paragraph context in a sample of 2400 paragraphs drawn from 162 authors. 2100 different words were contained in the 8,000 original associations. A computer program, utilizing the shared meaning pairs, reduced these 2100 words to 242 "supra-generic" concepts to produce a more useful associational index. This index was then utilized to select and sequence paragraphs to form two representative multi-author manuscripts, which by our theory should simulate the unconscious strategy a single author follows from items stored in brain memory.

Major Findings: Each of the two quite different manuscripts could be read relatively smoothly despite the fact that 75 percent of the paragraphs came from different authors.

Proposed Course: To proceed further with theoretical development relating to establishing an operational "prosthesis for the brain" in the domain: "The influence of the built environment on the health and lives of people."

Significance to Biomedical Research and the Program of the Institute: This direction of inquiry can culminate in a more effective way of interrelating the insights developed by many persons. To the extent that this expectation is realized it will contribute to the continuation of the evolutionary process by enabling more rapid and more complex interrelationships among ideas than is possible alone from biological brain processes and the process of interpersonal communication developed through culture. The negentropy level of this part of the universe will be increased as a result of a greater amount, diversity, and complexity of information having survival utility.

Publication: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00881-24 LCM

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Intermediary Energy Metabolism in Mammalian Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. Kaufman	Research Chemist	LCM NIMH
OTHER:	T. Nelson	Staff Fellow	LCM NIMH
	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has taken a new direction which includes studies on the origin, mode of action, and metabolism of γ-hydroxybutyrate, (GHB), a compound which is a normal constituent of mammalian brain and which is of increasing pharmacological interest. When administered in pharmacological doses, GHB produces a flattening of the EEG, a reversible trance-like state and a profound depression of cerebral glucose utilization. This compound is being used clinically in Europe as an anesthetic adjuvant. Preliminary trials indicate that it is effective in the treatment of narcolepsy and may be of use in the treatment of stroke.

GHB, and an enzyme capable of metabolizing it, have been found in tissues such as liver and kidney as well as in brain. This enzyme, an $NADP^+$-linked alcohol oxido-reductase which interconverts GHB and succinic semialdehyde, has been purified from hamster liver and partially purified from hamster brain. Studies completed on this enzyme include substrate specificity, enzyme kinetics, molecular weight determination, and inhibition studies with the anticonvulsant agents, diphenylhydantoin, amobarbital, and valproate.

Project Description:

This project is directed at understanding the role of naturally-occurring γ-hydroxybutyrate as well as the metabolic pathways involved in its synthesis and degradation. Since the compound is also used as a drug, other important questions arise concerning its pharmacological actions. In addition to the pharmacological effects already mentioned (flattening of the EEG, trance-like state and depression of cerebral glucose utilization) it has recently been reported that γ-hydroxybutyrate increases the survival of animals which have been made hypoxic. The authors report that the blood and brain tissues of animals which have received γ-hydroxybutyrate showed marked changes in the levels of some intermediates in carbohydrate metabolism as well as changes in levels of high energy phosphate compounds. These studies have led to the proposal that γ-hydroxybutyrate may be useful in the treatment of stroke. Because γ-hydroxybutyrate is a compound which is used clinically, and because it is a naturally-occurring compound, we feel that it is important to understand not only pathways of metabolism of γ-hydroxybutyrate, but also the factors which control these pathways.

We have recently found that the oxido-reductase which catalyzes the oxidation of GHB also catalyzes the reduction of D-glucuronate to L-gulonate. This is an important step in the pathway leading from UDP-glucose to entire L-ascorbate or to L-xylulase and the pentose pathway. We have recently investigated the effect of both L-gulonate and of D-glucuronate on the rate of oxidation of γ-hydroxybutyrate. In the presence of limiting concentrations of NADP$^+$ or with limiting concentrations of NADP$^+$ and inhibitory concentrations of NADPH, D-glucuronate can increase the rate of oxidation of GHB by up to eight fold. Since this enzyme is a soluble enzyme found in the cytosal and since the cytosal of most tissues contains low concentrations of NADP$^+$ as well as relatively high concentrations of NADPH, D-glucuronate may be an important factor in controlling GHB metabolism *in vivo*.

The physical and chemical properties of this enzyme are also being investigated. Treatment of the enzyme with a reducing agent such as dithiothreotol produces marked inhibition of activity. This inhibition can be at least partially reversed by the addition of an oxidizing agent such as hydrogen peroxide. We are attempting to correlate this effect with the effect of these compounds on the physical state of the protein.

A study on the purification and characterization of an NADP$^+$-linked alcohol oxido-reductase capable of interconverting γ-hydroxybutyrate and succinic semialdehyde has been completed and published. This enzyme has been purified 300-fold and found to exhibit a single band on gel electrophoresis. The protein is a monomer with a molecular weight of $\sim$ 31,000. The enzyme is inhibited by two anticonvulsant agents, amobarbital and diphenylhydantoin, as well as by valproate, a drug used in the treatment of epilepsy. Pyrazole, the specific inhibitor of alcohol dehydrogenase (NAD$^+$-linked) does not inhibit this enzyme. Diethyldithiocarbamate, the reduced form of the drug antabuse, as well as KCN, both metal-chelating agents, inhibit the enzyme. This suggests that the enzyme may contain either copper or zinc as an integral part of the protein. This possibility is currently being investigated.

In vitro, alcohol dehydrogenase (NAD^+-linked) (ADH) is also capable of catalyzing the interconversion of γ-hydroxybutyrate and succinic semialdehyde (SSA). The Km for γ-hydroxybutyrate with alcohol dehydrogenase is, however, an order of magnitude higher than the Km with the $NADP^+$-linked oxido-reductase. In vivo studies on the half-life of $[^{14}C]$γ-hydroxybutyrate (in plasma) in the presence and absence of various inhibitors have been carried out in an effort to assess the contribution of these two enzymes to the metabolism of γ-hydroxybutyrate in the whole animal. Results of these studies show that pyrazole, a potent inhibitor of ADH, does not affect the half-life of γ-hydroxybutyrate. The same concentration of pyrazole produced severe inhibition of ethanol metabolism in the whole animal. This suggests that ADH is probably not the enzyme responsible for the oxidation of γ-hydroxybutyrate in the whole animal. Preliminary results show that two compounds which inhibit the oxidation of γ-hydroxybutyrate catalyzed by the $NADP^+$-linked oxido-reductase, dilantin and L-gulonate, increase the half-life of γ-hydroxybutyrate in the plasma (indicating a decreased rate of metabolism). These results lend support to a role for the $NADP^+$-linked alcohol oxido-reductase in the metabolism of γ-hydroxybutyrate in the whole animal.

Changes in the levels of various intermediates in carbohydrate metabolism, most notably lactate, as well as changes in high energy phosphate compounds following administration of γ-hydroxybutyrate, have been reported. We are currently investigating the biochemical basis for the changes which have been observed both in normoxic and in hypoxic animals.

Identification of γ-hydroxybutyrate in tissues other than brain is a project which is nearing completion. It has been found that both liver and kidney contain higher levels than those observed in brain tissue. The compound has been detected in a number of other tissues, although the levels are not as high as those found in kidney and liver.

The behavioral effects of exogenously administered γ-hydroxybutyrate are very similar to those seen in hibernation, a phenomenon that is still not understood. The possibility that γ-hydroxybutyrate may be involved in hibernation is being examined in this laboratory. Preliminary studies in the hibernating hamster indicate that there is a significant increase in the level of the enzyme that oxidizes γ-hydroxybutyrate in brain but not in liver. These studies will be continued and will be extended to the measurement of γ-hydroxybutyrate levels in brain in hibernation. Progress in this area will be greatly facilitated by the recent acquisition of a gas chromatographic unit with a nitrogen-detector, which should simplify the assay of γ-hydroxybutyrate.

A new spectrophotometric assay for the detection of γ-hydroxybutyrate has been devised. It is based on the use of the purified $NADP^+$-linked alcohol oxido-reductase and the synthetic co-factor, 3-acetylpyridine-adenine dinucleotide phosphate. The substitution of the synthetic co-factor shifts the equilibrium of the reaction $GHB + NADP^+ \quad SSA + NADPH + H^+$ far to the right so that the GHB may be estimated by the amount of reduced co-factor which has been produced. We anticipate that this assay will prove useful in the estimation of blood and tissue levels of γ-hydroxybutyrate.

This project will have a continued interest in ketone body metabolism in normal as well as in pathological states. D-β-Hydroxybutyric acid dehydrogenase catalyzes the reversible interconversion between D-β-hydroxybutyrate and acetoacetate, the two ketone bodies found primarily in the liver when fatty acid utilization is increased, as, for example, in diabetes, starvation, high-fat diet, and various states leading to ketosis. Acetoacetate is the primary ketone body formed from catabolism, and it is converted to D-β-hydroxybutyrate by the action of the enzyme. Once formed, however, D-β-hydroxybutyrate cannot be used directly in any of the tissues; it must first be converted back to acetoacetate by the action of the same enzyme. This raises the interesting question of what role the enzyme plays. Why does the body not utilize the directly-formed acetoacetate rather than convert it first to D-β-hydroxybutyrate only to oxidize it back to acetoacetate again to utilize it? There is evidence in the literature to suggest that excessive concentrations of acetoacetate in the tissues may be toxic, particularly in brain which has recently been shown to utilize ketone bodies in direct proportion to their levels in the blood. The possibility existed that the role of the enzyme is protective to keep the acetoacetate concentrations low by storing it, in a sense, as D-β-hydroxybutyrate. It is noteworthy that there are several metabolic conditions characterized by ketosis and coma. Diabetic coma is one example, and recently Reye's Syndrome has been described. This is a frequently fatal disease of childhood which occasionally follows a viral infection, particularly influenza. It is characterized by fatty degeneration of the liver, ketosis, and coma.

Examination of the metabolic pathways and the enzymes responsible for the metabolism of ketone bodies in brain suggests possible mechanisms by which forced excessive use of ketone bodies in brain may lead to depressed cerebral functional activity. The ketone bodies enter the Krebs Cycle after their prior conversion to acetylCoA. The pathway is the conversion of β-hydroxybutyrate to acetoacetate which then displaces succinate from succinylCoA to form acetoacetylCoA. The acetoacetylCoA is then converted to acetylCoA and enters the Krebs Cycle. For every acetoacetate molecule thus metabolized, there is one succinylCoA degraded. SuccinylCoA is required for the formation of GTP which is the precursor of cyclic GMP. Excessive use of ketone bodies can theoretically, therefore, lead to depletion of available GTP and, therefore, also cyclic GMP. There is now a considerable body of evidence that cyclic GMP is the second messenger in the action of the neurotransmitter, acetylcholine. Its depletion can be expected to result in depression of cholinergic functions in brain and lead possibly to depressed functional activity and even coma. The possibility that ketosis may lead to coma by depletion of brain cyclic GMP is currently being studied in this laboratory. A radioimmune assay for cyclic GMP has been established and preliminary results do indeed suggest that in diabetic acidosis there is a depression of cyclic GMP in brain. These studies will be more aggressively pursued during the next year.

Proposed Course:

This project will continue on the course which has been outlined. It will include: 1) further kinetic studies with $NADP^+$-linked alcohol oxido-reductase; 2) determination of the presence or absence of a metal in this enzyme;

3) *in vivo* studies to determine the effect of various drugs, normal metabolites and alterations in physiological state on the metabolism of GHB; 4) continued investigation of GHB metabolism in the hibernating animal; 5) investigation of the interaction of GHB with other pathways of carbohydrate metabolism; 6) study of the biosynthetic pathway for GHB; 7) developmental studies on the levels of GHB and of the $NADP^+$-linked alcohol oxido-reductase in brain and other tissues. 8) This project will have a continued interest in ketosis, β-hydroxybutyrate metabolism and the relationship between ketosis and cyclic GMP levels in brain.

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PROJECT NUMBER (Do NOT use this space)

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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00882-13 LCM

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Studies on Regional Cerebral Circulation and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH
OTHER:			
	D. Ingvar	Visiting Scientist	LCM NIMH
	C. Kennedy	Guest Worker	LCM NIMH
	M. Miyaoka	Visiting Associate	LCM NIMH
	C. S. Patlak	Chief, Theoretical Statistics & Math.Br.	B NIMH
	K. Pettigrew	Mathemat./Stat., Theoret. Stat. & Math. Br.	B NIMH
	M. Kadekaro	Guest Worker	LCM NIMH
	S. Suda	Visiting Fellow	LCM NIMH
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COOPERATING UNITS (if any) T.Duffy, Cornell U., NYC; M.Phelps & D.Kuhl, UCLA, Los Angeles; W.Bunney & A.Pert, Adult Psychiatry Br.,NIMH; R.Post, Adult Psychiatry Br., NIMH; M.Mishkin, Lab. of Neuropsychology, NIMH; J.C.Gillin, Lab. of Clin. Psychopharmacology, DSMR, NIMH, J.DiMicco, Lab. of Clinical Sci.,NIMH; S.Rapoport, Lab. of Neurobiology, NIA, NIH.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method has been developed for the quantitative determination of the rates of local glucose consumption in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of $[^{14}C]$deoxyglucose as a tracer for glucose flux through the hexokinase step. Local $[^{14}C]$deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping functionally, and therefore metabolically, linked structures in the CNS, the method is being used to map the functional visual pathways in the monkey. It is also being used to study alterations in the energy metabolism of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states.

Professional Personnel Engaged on the Project (continued)

F. Orzi	Visiting Fellow	LCM NIMH
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A. C. Tannenbaum	Research Biologist	LCM NIMH

Project Description:

Previous work in this Laboratory several years ago led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{14}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate kinetically between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [^{14}C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the cerebral tissues and the hexokinase kinetic constants (V_{max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six individual ones. Further mathematical analyses revealed how to design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.483, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey and the cat; the values have been found to be 0.344 and 0.41, respectively. In collaboration with T. Duffy of the Department of Neurology, Cornell University, the lumped constant has been measured in the dog and found to be 0.56.

All the theoretical and technical problems were solved, and the method has now been completely operative for the last three years. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [^{14}C]deoxyglucose is injected, and arterial plasma concentrations of [^{14}C]deoxyglucose and glucose are measured from the time of injection till 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [^{14}C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped constant, local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer.

The original operational equation was valid only under conditions in which the plasma glucose level remained constant throughout the experimental period. This requirement was cumbersome because many experimental conditions cause the plasma glucose level to vary. A new operational equation was derived which does not have this restriction, but it requires knowledge of the half-life of the free glucose pool in the tissue. A method was, therefore, developed which measures the half-life of the free glucose pool for the first time. The method has been used in the rat, and the half-life has been found to average 1.2 min in the conscious state, 1.8 minutes in the anesthetized state, and to vary in proportion to the plasma glucose level. Aside from the usefulness of this method as an independent research tool, it has now made it possible to employ the [^{14}C]deoxyglucose technique in conditions in which the plasma glucose concentration varies during the procedure. This new method for the measurement of the turnover of the free glucose pool of brain has been developed to the point of general usefulness in its own right and published in 1980.

The [^{14}C]deoxyglucose method has been applied to normal conscious and anesthetized rats and conscious monkeys. The values obtained cannot be directly compared to any other data in the literature since measurement of local cerebral glucose consumption has never previously been possible, but the average of the local values is very close to the reported values for overall average brain glucose utilization in those two species. Anesthesia has been found to depress cerebral glucose consumption throughout the brain. Carbon dioxide inhalation has been shown to depress local glucose consumption even though it increases cerebral blood flow. Local cerebral blood flow has been found in the normal animal to correlate very closely with local cerebral energy metabolism, a relationship which had been hypothesized but never previously proved. Increased local functional activity produced by electrical stimulation or by experimentally induced focal seizures following local penicillin injection results in markedly increased local glucose consumption in appropriate regions of the CNS. The studies with experimental focal epilepsy have established the existence of a "mirror focus" contralateral to the epileptogenic focus. These results have established once and for all the often debated but never previously proved fact that energy metabolism in the CNS is closely coupled to functional activity as it is in all other tissues.

Studies have been completed on the effects of normal aging in rats from young adulthood of 4-6 months to old age of 26-36 months. The results demonstrate decreases in glucose utilization in selective regions of the brain. There is a general reduction in activity and the extent of the neuropile through most of the cerebral cortex. There are decreases in the caudate-putamen system. There is a decrease in the parietal cortex. The most prominent changes, however, are in the primary sensory systems, mainly those involved with vision and audition. These entire systems were markedly depressed metabolically, suggesting that there was a depression of sensory input due to peripheral sense organ failure, i.e., the eyes and the ears. Such results might suggest that major depressions in some brain functional activities may be the result of essentially deafferentations or separation from the environment because of functional losses in the peripheral sense organs. Studies in progress, in which dopaminergic systems are activated by administration of the dopamine-agonist, apomorphine, indicated age-related depression in responsivity of the nigrostriatal system, perhaps due to losses in dopamine receptors.

Studies have been completed on the effects of amphetamine in the rat. This dopaminergic agonist produces pronounced increases in glucose utilization in selected structures of the brain, in particular the components of the extrapyramidal dopaminergic motor system, i.e., caudate nucleus zona compacta and zona reticulata of the substantia nigra, etc. A markedly affected structure was the sub-thalamic nucleus, which had previously not been suspected to be dopaminergic, but in follow-up studies based on those of this laboratory, has been found to contain an active dopamine-sensitive adenylcyclase. Similar studies have been carried out on the effects of another type of dopamine-agonist, apomorphine, which has been found to have similar effects on local cerebral glucose utilization. Studies have been completed in collaboration with Drs. Agu Pert and William Bunney of the Adult Psychiatry Branch, NIMH, on the supersensitization of dopaminergic synapses by chronic administration of the dopamine-blocking drug, haloperidol. The results thus far clearly demonstrate metabolic correlates of the super-sensitivity phenomenon. Studies of the actions of apomorphine also led to the demonstration of dopaminergic synapses in the retina in the neural pathway from the retina to the superior colliculus.

Studies on the effects of the β-adrenergic blocking agent, propranolol and the α-adrenergic blocking drug, phentolamine, have led to surprising results. These drugs have directly opposite effects selectively on the auditory system of the rat. Propranolol, which is presently being widely touted as an anti-hallucinogenic agent in schizophrenia and other psychoses, was found to depress markedly the rates of glucose utilization throughout the entire auditory system from the cochlear nucleus to the auditory cortex. In contrast, phentolamine, which has mild depressant effects in most other parts of the brain, has exactly the opposite effects in the components of the auditory system, namely a pronounced increase in glucose utilization. Studies have been completed extending the investigations to other adrenergic agonistic and antagonistic drugs. The results of these studies are currently being analyzed and prepared for publication.

In the course of the development of the method it became apparent that even without complete quantification, the autoradiographs represented pictorial displays of the relative rates of energy consumption in all parts of the brain. For example, they demonstrated clearly that the synapse-rich areas, e.g., layer 4 of the neuropile in the cerebral cortex, are the most metabolically active areas of the cortex. It also became apparent that inasmuch as local glucose consumption changes in response to changes in functional activity, regions of the brain which were turned on or off functionally and metabolically in altered physiological states could be mapped simultaneously throughout the brain. The efficacy of this technique of mapping by "evoked metabolic response" has been demonstrated in the visual and auditory systems of the rat and monkey. All the regions involved in visual functions have, for example, been shown to alter activity and be displayed on the autoradiographs after occlusion of vision of one eye and stimulation of the other. By this technique the visual cortical locus of the representation of the blind spots of the visual fields has been demonstrated in the monkey and its shape and dimensions defined. The resolution of the method is surprisingly good. It has clearly demonstrated the ocular dominance columns described by Hubel and Wiesel, which are believed to represent the structural and functional organization of the visual cortex responsible for the integration of the two images from the two eyes in animals with binocular vision. Application of the method to the newborn monkey has demonstrated that these columns are already organized at birth. Patching of one eye in the newborn for 3 months leads to a reorganization of the visual cortex so that the portions representing the blocked eye are taken over by the portions of the visual cortex subserving the open eye. Such plasticity is not present in the mature monkey brain.

As part of a general program of research on the mapping with [^{14}C]deoxyglucose of the central pathways involved in the control of autonomic functions, studies have been carried out on the central pathways of the gastrosecretory response induced by insulin hypoglycemia. These studies located 5 regions of the brain which respond with increased metabolic activity to reductions of plasma glucose concentration within the physiological range. Three of these structures are part of the pathway ending in the vagal innervations of the gastric mucosa. The other two may be associated with other physiological responses to hypoglycemia. These studies demonstrated the usefulness of the method for studying central autonomic processes, and similar studies are now in progress on the control of blood pressure.

The method has also been used to study the visual systems of the albino and pigmented rats. It has shown that the visual system of the albino rat is more sensitive than that of the pigmented rat at very low light levels, but is overwhelmed or "fatigued" at higher light levels which cause a reduction in local glucose utilization in the visual structures. These studies have also demonstrated a linear relationship between the rate of local glucose utilization in central visual structures and the log of the intensity of retinal stimulation, indicating that local metabolic rate in the nervous system, like behavioral manifestations, obeys the Weber-Fechner Law.

Studies during the past year on the superior cervical ganglion have demonstrated that the local rate of glucose utilization is closely coupled to the electrical activity. Electrical stimulation of the pre-ganglionic input to this ganglion results in increased glucose utilization in the ganglion, and the rate of glucose utilization is directly and linearly related to the spike frequency. Antidromic stimulation of the efferent nerves from this ganglion stimulates glucose utilization in the cell bodies of origin of the efferent nerve. The locations of the cell bodies are clearly visualized in the autoradiographs, and this combination of antidromic electrical stimulation with the deoxyglucose method offers promise as a technique to find the origin of neural pathways in the nervous system.

Studies are in progress to determine the mechanisms by which glucose utilization is coupled to functional activity. Increased functional and electrical activities are known to increase extracellular K^+, and increased extracellular K^+ may stimulate energy metabolism by activation of the Na^+,K^+-ATPase. Experiments have been carried out in which KCl is applied to the parietal cortex trans-durally by its topical application through a hole in the skull or directly on the pial surface of the cortex. Such application of the KCl markedly stimulates glucose consumption in the underlying cortex, probably by direct action, while depressing metabolic activity in subcortical structures, probably by reflex or neurogenic reduction of functional activity. This treatment is known to cause the phenomenon of spreading cortical depression during which the DC potential of the cortex is shifted negatively. Studies during the recovery from spreading depression (i.e., immediately following the return of the DC potential to normal) have demonstrated that there is still increased cortical glucose utilization, but it is distributed in a columnar pattern traversing the thickness of the cortex. These results give additional support to the accumulating evidence of the columnar organization of functional activity in the cerebral cortex.

In order to determine whether the activation of glucose utilization by increased electrical and/or functional activities or by increased extracellular K^+ was due to stimulation of Na^+,K^+-ATPase activity, studies have been carried out on the posterior pituitary which secretes vasopressin under control of the hypothalamo-hypophysial tract. Studies in rats under conditions of salt-loading, which stimulates this pathway and vasopressin secretion, demonstrated that glucose utilization in the posterior pituitary is markedly stimulated under these conditions. When the posterior pituitary was electrically stimulated in vitro, its glucose utilization was also increased, and this increase could be blocked by ouabain, a specific inhibitor of the Na^+,K^+-ATPase. Glucose utilization of the posterior pituitary in vitro could also be stimulated by veratridine, an agent that opens the Na^+ channels in the cell membranes and increases Na^+ content in the cells. The stimulation of glucose utilization was also blocked by ouabain and by tetrodotoxin, an agent that blocks the Na^+ channels. These results establish that the stimulation of glucose utilization by increased functional and/or electrical activities is mediated by activation of the Na^+,K^+-ATPase.

The method has also been used to study the acute and delayed effects of hyper-osmolar opening of the blood-brain barrier. The barrier was opened by unilateral carotid arterial injections of hyper-osmolar mannitol. Shortly after the barrier is opened, there is metabolic evidence of punctate regions of seizure-like activity on the side of the barrier opening. The effect is apparently reversible, for those areas of markedly increased metabolic activity return to normal after a period of an hour or more following opening of the barrier.

The regional localization obtained with the $[^{14}C]$deoxyglucose method is achieved by the use of quantitative autoradiography. The autoradiographs provide pictorial representations of the relative, not the actual, rates of glucose utilization in all structures of the brain. They are ordinarily subjected to manual densitometric analysis from which local ^{14}C concentrations are derived and used in the operational equation to compute the actual rates of local glucose utilization. We have recently developed a computerized image-processing system to analyze and transform the autoradiographs into color-coded pictorial maps of the actual rates of glucose utilization throughout the entire CNS. The autoradiographs are scanned automatically by a computer-controlled scanning microdensitometer which measures the optical density of each spot, 25-100 μm, in the autoradiograph. These optical densities are stored in a computer, converted to tissue ^{14}C concentrations on the basis of the optical densities of calibrated ^{14}C plastic standards, and then converted by the computer to actual local rates of glucose utilization by solution of the operational equation. Colors are assigned to narrow ranges of the rates of glucose utilization, and the autoradiographs are then displayed on a color monitor in color along with a calibrated color scale for identifying the rate of glucose utilization in each spot of the autoradiograph from its color. These pictures are, therefore, complete color maps of the actual rates of local glucose utilization precisely localized in each 25-100 μm region of the CNS. Work is in progress to develop computerized techniques to reconstruct color maps of the entire brain three-dimensionally from the digitized autoradiographs.

Recent technological developments with computerized axial tomography have made it possible to scan the human brain for local tissue concentrations of positron-emitting isotopes. Experiments have, therefore, been carried out with [^{18}F]fluoro-deoxyglucose, a positron-emitting analogue of [^{14}C]deoxyglucose, and the results demonstrate that it can be used in place of [^{14}C]deoxyglucose as the tracer for the deoxyglucose method. This now makes it possible to use the method in man. In collaboration with Dr. M. Reivich of the University of Pennsylvania, and M. Phelps and D. Kuhl of UCLA, the method has been adapted for human use. The NINCDS has procured a scanner which is now operational. This instrument will be available for our use, and we are about to initiate studies of psychotic states, aging, and dementia in man.

Several lines of further investigation are planned in this project. One line is methodological by which it is hoped to improve the resolution of the method to the microscopic or single cell level. Another line is the exploitation of the [^{14}C]deoxyglucose method at its present state in studies of the effects of aging, psychoactive drugs, metabolic comas, development and maturation of the brain, and hormones such as thyroxine known to have important actions on the maturation of the brain.

APPENDIX

Contract: Contract No. 278-79-0004 (IR) University of Montreal
Contract Title: Deoxyglucose Method for Measurement of Local Cerebral Glucose Utilization

The present deoxyglucose method achieves a spatial resolution of 100 μm. This is adequate to measure glucose utilization only macroscopically in the structural components of the nervous system. For many neurophysiological and neuroanatomical studies it is necessary to study single cells or even components of cells. To achieve this requires extension of the resolution of the method to the light and electron microscopic levels. The theoretical basis of the method allows for such resolution, but technical problems associated with autoradiography of water-soluble tracers make this a difficult problem.

The Laboratory of Cerebral Metabolism lacks the physical facilities and resources as well as the necessary experience and expertise to carry out the necessary neuroanatomical procedures required for this technological development.

A contract was, therefore, made with Drs. M.H. Des Rosiers and L. Descarries of the University of Montreal to undertake the studies needed to accomplish this development. Dr. Des Rosiers was trained in all the intricacies of the deoxyglucose method in our Laboratory; indeed, he was a member of the team that developed the method. Dr. Descarries directs a neuroanatomical laboratory which has had extensive experience with high resolution autoradiography. They have made considerable progress toward the achievement of microscopic resolution. They utilize [^{3}H]deoxyglucose and perfuse the brain *in vivo* with glutaraldehyde and osmic acid. They then remove the brain, dehydrate it with anhydrous acetone, and embed it in Epon. This procedure manages to preserve the labeled compound in the tissue without moving it from its original site. The embedded brain is then sectioned for autoradiography. It is necessary to use dipping emulsion for the autoradiography, but dipping emulsion is water-based and may wash out the label. Work is progressing on finding a technique by which the dipping emulsion will not displace the label. When this goal is achieved, the method should be close to becoming operational for studies at the cell and sub-cellular levels.

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21. Sokoloff, L.: Metabolic mapping of local functional activity in the central nervous system. Trans. Amer. Neurol. Assoc. (in press) 1980.

22. Batipps, M., Miyaoka, M., Shinohara, M., Sokoloff, L., and Kennedy, C.: Comparative rates of local cerebral glucose utilization in the visual system of conscious albino and pigmented rats. Neurology (in press) 1980.

23. Smith, C.B., and Sokoloff, L.: The energy metabolism of the brain. In The Molecular Basis of Neuropathology, (A.N. Davison, and R.H.S. Thompson, eds.), London, Edward Arnold, Ltd., (in press) 1980.

24. Sokoloff, L., and Smith, C.B.: Effects of normal aging on regional cerebral glucose utilization. International Symposium on Experimental and Clinical Methodologies for the Study of Acute and Cerebro-Vascular Diseases. Paris, (in press) 1980.

25. Savaki, H., Davidsen, L., Smith, C., and Sokoloff, L.: Measurement of free glucose turnover in brain. J. Neurochem. (in press) 1980.

26. Kennedy, C., and Sokoloff, L.: The [^{14}C]2-deoxyglucose method for measuring local cerebral glucose utilization: theory and practice. In Current Trends in Morphological Techniques, CRC Press (in press) 1980.

27. Sokoloff, L.: Theory, procedure, and applications of the radioactive deoxyglucose method for the measurement of local glucose utilization in the central nervous system. In Advances in Neurochemistry (in press) 1980.

28. Goochee, C.F., Rasband, W.S., and Sokoloff, L.: A computer assisted image processing system for the analysis of autoradiographs of cerebral metabolic activity. Journal of Medical Systems (in press) 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00886-03 LCM

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Circadian Rhythmicity of Glucose Utilization in the Rat Suprachiasmatic Nucleus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. B. Smith	Staff Fellow	LCM NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cerebral Metabolism, NIMH

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study the autoradiographic 2-deoxy-D[^{14}C]glucose method of determining glucose utilization was applied in an attempt to demonstrate a circadian rhythm of an intrinsic property of the rat suprachiasmatic nucleus (SCN). It was found that (1) SCN glucose utilization could be entrained to a 24 hr environmental light-dark cycle and phase shifted 180° by reversing the external entraining cycle. (2) This metabolic rhythm appeared to persist in the absence of any external light-dark cues but it was arrhythmic after 10 days of constant light. (3) The effect of ambient darkness during the light phase of the entraining cycle appeared to "unmask" an underlying endogenous metabolic activity of the nucleus. (4) When animals maintained in constant darkness or constant light were exposed to either ambient light or darkness, respectively, the resulting pattern of altered SCN glucose utilization suggested that the sensitivity of the nucleus to ambient light during constant conditions may have been a function of the phase of an endogenous SCN oscillation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives. The suprachiasmatic nucleus (SCN) in the anterior hypothalamus has been proposed as the endogenous circadian oscillator in mammals. The evidence is based on the fact that a retino-hypothalamic pathway terminating in the SCN has been identified and appears to be necessary for the entrainment of circadian rhythms to the environmental light-dark cycle and that lesions of the SCN abolish a wide spectrum of overt circadian rhythms. In order to demonstrate that a directly measurable property of the SCN itself in intact (unlesioned) animals is affected by environmental light and exhibits circadian rhythmicity, the autoradiographic 2-deoxy-D-[^{14}C]glucose method was used to determine glucose utilization of rat SCN under various lighting conditions.

Methods. A total of 88 adult, male Sprague-Dawley albino rats, weighing between 300 and 400 grams each, were used in experiments conducted from October, 1977, to April, 1978. Rats were housed in individual activity-wheel cages in a room with controlled artificial lighting. During "light" phases, the cages were bathed in approximately 30 ft-c illumination from overhead fluorescent bulbs; during "dark" phases, less than 0.01 ft-c illumination was provided by two Kodak 15 watt safelights with red (1A) filters. Individual running-wheel activities were recorded.

Groups of rats were subjected to various lighting regimens before the determination of SCN glucose utilization. At least two hours after catheterization of the femoral vein and artery, animals were injected intravenously with 2-deoxy-D-[^{14}C]glucose while they were alert and restrained. After 45 min the animals were killed by an intravenous injection of sodium pentobarbital; and their brains were removed, frozen in 2-methylbutane (-40°C), and serial coronal sections 20 µm thick were cut through the region of the SCN. Sections were dried and autoradiographs prepared.

After autoradiography, the sections were stained with cresyl violet to determine the location and extent of the SCN. Optical densities of the SCN and other selected brain regions were read on a Photoscan System P-1000 densitometer (Optronics International, Inc.). Optical densities of the left and right SCN were recorded for each section along the 600 µm length of the nucleus, and mean glucose utilization of the entire nucleus was calculated.

Major Findings. The results of these experiments indicate that the rat SCN has properties consistent with a role as an endogenous circadian oscillator. The glucose utilization of the SCN can be entrained to a 24-hr environmental light-dark cycle (LD) and phase-shifted 180° by reversing the external entraining cycle (DL). Glucose utilization is high during the light phase and low during the dark phase. This metabolic rhythm appears to persist in a somewhat modified form in the absence of any external light-dark cues, i.e., during constant darkness or after bilateral enucleation. After 10 days in these constant conditions, the nucleus is metabolically active for a shorter time and at a lower level of glucose utilization during the subjective day than it had been during the light phase of either an LD or DL cycle. However, after 10 days of constant light, SCN glucose utilization appears arrhythmic and at an intermediate level. When animals in the "D" phase of an LD cycle are exposed to light, SCN glucose utilization increases from low levels to high values comparable to those observed during the "L" phase. On the other hand, dark exposure during the "L" phase results in a pattern of altered SCN glucose utilization similar to that observed during the subjective day in constant darkness or after bilateral enucleation. Dark exposure thus appears to "unmask" the endogenous activity of the nucleus. When animals in constant darkness or constant light are exposed to either light or darkness, respectively, the resulting pattern of altered SCN glucose utilization suggests that the sensitivity of the nucleus to ambient light during constant conditions may be a function of the phase of an endogenous SCN oscillation.

Proposed Course. All the results of this project have been published and the project terminated.

Publications:

1. Schwartz, W. J., Smith, C. B., and Davidsen, L. C., *In vivo* glucose utilization of the suprachiasmatic nucleus. In: *Biological Rhythms and Their Central Mechanisms*, Proceedings of Naito International Symposium, M. Suda, O. Hayaishi, and H. Nakagawa, (eds.), Elsevier/North-Holland Biomedical Press, pp. 356-367, 1979.

2. Schwartz, W. J., Davidsen, L. C., and Smith, C. B., *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *J. Comp. Neurol*. 189:157-167, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00887-03 LCM

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Extended Visual System of the Macaque Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Kennedy	Guest Worker	LCM NIMH
	M. Mishkin	Research Physiologist	LN NIMH
OTHER:	M. Miyaoka	Visiting Associate	LCM NIMH
	M. Ito	Visiting Associate	LCM NIMH
	S. Suda	Visiting Fellow	LCM NIMH
	K. Macko	Guest Worker	LN NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropsychology, NIMH

LAB/BRANCH

Laboratory of Cerebral Metabolism, NIMH

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.25	OTHER: 0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The deoxyglucose method is being applied to the monkey to advance knowledge regarding the parts of the brain which are involved in the processing of visual information. By measuring rates of local cerebral glucose utilization in animals during their performance of tasks involving different types of visual stimuli we anticipate learning which parts of brain are involved in such functions as discrimination, memory and motivation. Also by studying animals at various ages, information will be obtained regarding the maturation of the visual processing system.

Project Description:

The goal of the collaborative effort initiated in 1978 was to map regions of monkey brain which were responsive to visual stimulation. The deoxyglucose method has been shown to be sensitive to even small differences in functional activity, and it was hoped that it might be possible to shed light on such complex aspects of visual function as discrimination, memory, or even the mechanism by which the brain assigns a value judgement on the character of visual stimulation and then initiates a response to it. The procedure followed is to prepare animals so that one hemisphere is completely deprived of visual input. One optic tract is sectioned as is the corpus callosum and forebrain commissures. Because the intact brain functions symmetrically and therefore has equal metabolic rates in all homologous structures, the finding of right-left differences in metabolic rates in the surgically prepared animals serves to identify the visually responsive regions. The experiments to date have demonstrated that these include the striate cortex and entire expanse of the circumstriate and inferior temporal cortex as far forward as the temporal pole. Responding subcortical structures include the lateral and dorsal amygdala, ventral putamen, ventral claustrum and tail of the caudate. Outside of the stimulus-processing system and its subcortical projections, responses are also present in the superior colliculus, inferior and lateral pulvinar and lower bank of the inferior parietal lobule. In studies of young monkeys it has been found that many of these structures begin their participation in visual function within the first few weeks of life.

The development of computer-assisted image-processing has made possible a more detailed analysis of the data than had heretofore been possible. It permits one to obtain average values for glucose utilization for histologically specific cortical areas. Thus we have proceeded to compare the effect of a stimulus which has reward value with one (a geometric design) having no behavioral significance. Also we have compared the results in animals prepared with optic tract section alone with those which had had tract section plus section of corpus callosum and forebrain commissure. This permits an assessment of the contribution of partially processed stimuli from the opposite hemisphere.

The collaboration will continue through the year in order to extend the number of animals in each experimental category. This increment in number of experiments will allow the attribution of statistical significance to the differences found.

The Laboratory of Neuropsychology report on this project is number Z01 MH 02033-03 LN, titled Functional Mapping of Sensory Systems.

Publications:

1. Macko, K.A., Miyaoka, M., Kennedy, C., Sokoloff, L., and Mishkin, M., Commissural contributions to vision measured by the 2-deoxyglucose technique. Neurosci. Abstr. (in press) 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00889-01 LCM

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

A Method for the Determination of Local Rates of Protein Synthesis in Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Smith	Staff Fellow	LCM NIMH
OTHER:	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH
	C. Kennedy	Guest Worker	LCM NIMH
	C. S. Patlak	Chief, Theoretical Statistics & Math.Br.	B NIMH
	K. Pettigrew	Mathem/Stat.,Theor.Stat.& Math.Br.	B NIMH
	M. Miyaoka	Visiting Associate	LCM NIMH
	S. Suda	Visiting Fellow	LCM NIMH

COOPERATING UNITS (if any)
B. Agranoff, University of Michigan, Ann Arbor, Michigan
R. Collins, Washington University, St. Louis Mo.
M. Phelps, UCLA, Los Angeles, Calif.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. The method is based on the use of [1-^{14}C]-L-leucine as a tracer for the incorporation of leucine into protein. A model of the behavior of leucine and [1-^{14}C]leucine has been designed. By mathematical analysis of the kinetics of exchange of the amino acid between plasma and tissue and its incorporation into protein, an equation has been derived that defines the rate of amino acid incorporation into protein in terms of the time course of plasma specific activity, final tissue concentration of ^{14}C, and experimentally determined kinetic constants. Tissue concentrations of ^{14}C are determined by quantitative autoradiography.

The method is currently being applied to studies of development, cretinism, the visual system, seizures and regeneration.

Project Description:

A method is being developed for the estimation of local rates of protein synthesis in brain *in vivo*. This method is similar to the [^{14}C]deoxyglucose method in that it is based on the principles of enzymology as applied to the measurement of reaction rates with labeled tracers as substrates. In order to measure the rate of the reaction, one must know the amount of product formed in a given interval of time and the history of the specific activity of the precursor. In an *in vivo* experiment the precursor pool cannot be sampled and the specific activity determined directly. It is necessary, therefore, to design a model for the behavior of the precursor *in vivo* and by kinetic analysis of the model derive a relationship between the entire history of the precursor specific activity in the plasma (which can be sampled and measured directly), the integrated specific activity of the precursor pool in the tissue, and the rate of the reaction.

We have chosen [1-^{14}C]L-leucine as the radiolabeled tracer for this method because the ^{14}CO$_2$ derived from its metabolism is rapidly diluted in the pool of CO$_2$ and cleared from the tissue. In the model for the behavior of leucine and [1-^{14}C]leucine in the tissue, a homogeneous tissue compartment and no reutilization of either the labeled or the unlabeled amino acid are assumed. The model requires a steady state for protein and amino acid metabolism. By mathematical analysis of the kinetics of the exchange of the amino acid between plasma and tissue and its incorporation into protein, an equation has been derived that defines the rate of leucine incorporation into protein in terms of the following measurable variables: the time course of plasma specific activity, final tissue concentration of ^{14}C and experimentally determined kinetic rate constants.

The equation defines the procedure to be used and the variables to be measured. A pulse of [^{14}C]leucine (100 μCi/kg body weight) is administered intravenously, and the arterial plasma concentrations of labeled and unlabeled leucine are monitored for the duration of the experimental period. At 30-60 minutes the rat is killed by an intravenous injection of pentothal. The brain is removed, frozen and sectioned and local tissue concentrations of ^{14}C are determined by quantitative autoradiography.

The validity of the assumption that there is no reutilization of the unlabeled leucine remains to be proven. *In vitro* studies (Robertson and Wheatley, 1979) have shown that the precursor pool for protein synthesis is in the extracellular space. Degradation of protein takes place within the cell, probably within lysosomes. This compartmentalization would preclude mixing of the leucine derived from protein degradation with the precursor amino acid pool for protein synthesis. *In vivo*, however, because the extracellular space is small, this mixing might occur outside the cell. We are currently devising *in vivo* experiments to test this extrapolation from *in vitro* results.

We have determined rates of protein synthesis in a number of brain regions. We find a wide range of values from 1.5 nmoles leucine/g of tissue/min in white matter to 20 nmoles/g/min in some hypothalamic nuclei (e.g., the supraoptic nuclei). In general, brain regions that are rich in nerve cell bodies, such as the pyramidal cell layer in the hippocampus and nerve nuclei such as the dorsal motor nucleus of the vagus, have high rates of protein synthesis as compared to either white matter or regions composed largely of nerve terminals, dendrites, synapses, and axons, such as the caudate nucleus, thalamus and cortex. The value that we have obtained for cortex (5.0 nmoles leucine/g/min) compares favorably with values obtained by Dunlop et al. (1975) of 4.7 nmoles valine/g/min with a completely different method.

We have embarked on several studies of the effects of some treatments or conditions on local rates of protein synthesis. The purpose of these studies is to test the sensitivity of the method to detect changes in local rates of protein synthesis. Studies of the effect of injury to the hypoglossal nerve have been carried out in collaboration with Dr. B. Agranoff. These experiments have shown that three days after cutting the hypoglossal nerve there is a 20% increase in protein synthesis in the nucleus of the XII nerve. Experiments conducted by Dr. R. Collins with our method have shown that bicuculline-induced seizures produce a reduction in protein synthesis. Other studies in progress in this laboratory include studies of the local rates of protein synthesis in brain during development and in cretinism. Dr. C. Kennedy is using the method to study the development and plasticity of the visual system in the newborn monkey.

Proposed Course: Plans are now being formulated in collaboration with Dr. Michael Phelps of the Division of Nuclear Medicine, UCLA, an organization that has a functional positron-emission tomographic laboratory, to adapt the method for use in man. In the Laboratory of Cerebral Metabolism, the method will be used to carry out or complete studies in thyroid hormone influences on brain development, plasticity of the visual system of primates, and slow-wave, and, perhaps, REM sleep, in the monkey.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00900-24 LCM

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on Myelin and Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Martenson	Research Chemist (Biochem.)	LCM	NIMH
Other:	G. E. Deibler	Chemist	LCM	NIMH
	M. L. Pedersen	Biologist	LCM	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three avenues of work are being pursued: (1) specific cleavage of the myelin basic protein by enzymes to obtain fragments for biochemical and immunological studies, (2) theoretical studies aimed at elucidating the secondary structure of the basic protein, and (3) studies aimed at defining the nature of the interactions between the basic protein and lipid molecules in the myelin membrane. These studies should contribute to a better understanding of how the basic protein stabilizes the myelin lamellar structure and why the protein is a powerful autoantigen.

P-S-6040
(Rev. 10-76)

Project Description:

In previous work we have been able to obtain a number of relatively small peptides from the rabbit basic protein by controlled peptic cleavage at pH 6.0. We are currently preparing larger peptic peptides of the protein, so that it will be possible to identify all products of the protein that are sequentially produced by the action of pepsin. By comparing the spectra of peptic peptides produced from the protein in solution and in isolated myelin, we hope to be able to determine which of the potentially susceptible bonds, if any, are inaccessible to the enzyme when the basic protein is in its native environment, complexed with lipids. Since pepsin cleaves primarily between hydrophobic residues, the results could provide important information regarding the degree to which hydrophobic residues penetrate the lipid bilayer of the myelin membrane.

An investigation into the probable secondary structure of the myelin basic protein was carried out by the application of three procedures currently in use to predict the secondary structures of proteins from knowledge of their amino acid sequences. In order to increase the accuracy of the predictions, the amino acid substitutions that occur in the basic protein from different species were incorporated into the predictive algorithms. It was possible to locate regions of probable α-helix, β-structure, β-turn, and unordered conformation (coil) in the protein. One of the predictive methods introduces a bias into the algorithm to maximize or minimize the amounts of α-helix and/or β-structure present; this made it possible to assess how conditions such as pH and protein concentration or the presence of anionic amphiphilic molecules could influence the protein's secondary structure. The predictions made by the three methods were in reasonably good agreement with one another. They were consistent with experimental data, provided that the stabilizing or destabilizing effects of the environment were taken into account. According to the predictions, the extent of possible α-helix and β-structure formation in the protein is severely restricted by the low frequency and extensive scattering of hydrophobic residues, along with a high frequency and extensive scattering of residues that favor the formation of β-turns and coils. Neither prolyl residues nor cationic residues *per se* are responsible for the low content of α-helix predicted in the protein. The principal ordered conformation predicted is the β-turn. Many of the predicted β-turns overlap by two residues; such overlaps would cause the peptide backbone to oscillate in a sinusoidal manner, generating a flat, pleated sheet-like structure. Cationic residues located in these structures would appear to be ideally oriented for interation with lipid phosphate groups located at the cytoplasmic surface of the myelin membrane. An analysis of possible and probable conformations that the triproline sequence could assume questions the popular notion that this sequence produces a hairpin turn in the basic protein.

In order to gain some understanding of the relative importance of electrostatic and hydrophobic interactions in holding the basic protein between the myelin membranes, we have been studying the factors involved in selective removal of the basic protein from isolated myelin. We have found that 0.1M HCl or NaCl at neutral pH are effective in removing only a fraction of the basic protein. The amount of basic protein extracted by salt solutions increases with salt concentration up to about 0.5M, indicating that this fraction of the protein is electrostatically bound. The efficiency of extraction with 0.1M HCl and 0.5M NaCl are comparable, suggesting that the pool of basic protein extracted by the two solvents is identical. We are currently examining the "refractory" basic protein by using the same media containing urea and solutions of urea alone to assess the possibility that most of the basic protein is held in place primarily through hydrophobic interactions or is inaccessible to the extracting medium unless the lipid bilayers are extensively disorganized. One possibility that will be examined is that the salt-extractable basic protein is located primarily in "heavy" myelin, which consists of uncompacted myelin membranes, whereas the refractory basic protein is located primarily in "light" myelin, which consists of compact myelin lamellae.

Publications:

Martenson, R. E.: Myelin basic protein: what does it do? In Kumar S. (Ed): Biochemistry of Brain. Pergamon Press, Oxford, 1980, pp. 49-79.

Martenson, R.E., Lüthy, V., and Deibler, G.E.: Cleavage of rabbit myelin basic protein by pepsin. J. Neurochem. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00901-25 LCM

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Immunologic Reactivity of Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH
Other:	J. R. Richert	Guest Worker	LCM	NIMH

COOPERATING UNITS (if any)
National Multiple Sclerosis Society
E. E. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.0	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transfer of EAE with BP-sensitized Lewis rat spleen and lymph node cells has been used to study immune mechanisms in the induction and suppression of EAE. Specifically, we have attempted to define the conditions under which culture with specific antigen activates BP-sensitized spleen and LN cells as well as those conditions which might generate antigen-specific suppressor cells in culture.

PHS-6040
(Rev. 10-76)

Project Description:

In studying mechanisms of activation and suppression of EAE lymphoid cells in the Lewis rat, we have found that both spleen cells and lymph node cells (LNC) from myelin basic protein (BP)-sensitized rats can be activated by culture with BP. Activated cells are capable of transferring disease 100-1,000 times more efficiently than cells that are not cultured. Cellular proliferation, measured by tritiated thymidine (^{3}H-TdR) uptake is also enhanced by addition of BP to cultured cells. The facility with which EAE can be transferred allows us to study in detail the events that lead to cellular activation and eventual disease production. It also permits a more extensive study of mechanisms of suppression that act on the effector arm of the immune response.

Cellular activation is antigen specific, in that it does not occur after culture with calf thymus histone (another basic protein used as a control antigen in culture) nor with tuberculin purified protein derivative (PPD) (an antigen to which the cells have been sensitized by virtue of the CFA in the sensitizing inoculum). Disease in recipient animals cannot be explained by carry-over of antigen from culture into the recipients. Removal of B cells from the unfractionated LNC further increases the effectiveness of transfer. We have so far been unable to detect a soluble factor (in culture supernatants or in homogenized cell preparations) capable of transferring EAE. Cells capable of being activated by culture with BP are found not only at the height of clinical disease, but also after recovery. This suggests that clonal deletion is not the mechanism of recovery from EAE, and indicates that cells capable of causing disease are actively suppressed in vivo.

Activation of sensitized LNC by BP can be inhibited by culture with dibutyryl cyclic AMP (Bt_2 cAMP). The inhibition is dose-dependent and is paralleled by a concommitant inhibition of lymphocyte proliferation. The cAMP-sensitive step appears to occur within the first 24 hours of culture. This inhibition is mimicked by isobutyl methylxanthine, a phosphodiesterase inhibitor which raises endogenous levels of cAMP. Attempts to suppress EAE in vivo with clinically safe phosphodiesterase inhibitors (such as aminophylline) are now underway in our laboratory.

We have investigated the theory that the mechanism of recovery from EAE involves the production of anti-idiotypic antibodies directed against an antigen recognition structure on the T-cell membrane. On two occasions adoptive transfer of EAE was inhibited when the BP-activated lymphoid cells were incubated with serum from rats that had recovered from EAE. However, this result has not been reproducible and, for the most part, our study is negative.

Proteases have been suggested as playing a crucial role in the destruction of myelin, and others have reported that EAE is inhibited by in vivo administration of various protease inhibitors. We have investigaged the possibility that proteases are involved in the processing of antigen by phagocytic cells before antigen is presented to lymphocytes. The possibility also exists that lymphocyte membrane proteases are involved in the activation process described above. To date we have been unable to prevent adoptive transfer of EAE by adding any of the following protease inhibitors to lymphoid-BP cultures: pepstatin A, trasylol, soybean trypsin inhibitor, leupeptin, epsilon amino caproic acid, and p-nitrophenyl guanidinobenzoate.

In an attempt to identify the antigen recognition site on BP-sensitized LNC, we have labelled BP-cultured cells with ^{35}S-methionine, and analyzed proteins in detergent extracts of these cells with SDS-polyacrylamide gel electrophoresis and autoradiography. Those proteins whose synthesis is enhanced by incubation with antigen are labelled most intensely. Our early results suggest that there may be several proteins in BP-activated cells not detected in Con A-activated normal LNC. There is also some evidence of quantitative differences in these proteins depending on whether BP-activated cells are incubated with PPD or BP. We are attempting to refine the identification of these membrane proteins with antisera raised against BP-activated lymphoid cells.

Publications:

Alvord, E. C., Jr., Shaw, C.-M., Hruby, S., and Kies, M. W.: Has myelin basic protein received a fair trial in the treatment of multiple sclerosis? Ann. Neurol. 6: 461-468, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00902-15 LCM

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. F. Driscoll	Guest Worker	LCM	NIMH
Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)
National Multiple Sclerosis Society
E. C. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In attempting to dissect the various cellular events responsible for the development of EAE, we have studied the effect of in vitro culture with antigen on the ability of BP-sensitized cells to transfer EAE to naive recipients. Two different cell compartments, peritoneal exudate cells, PEC, and lymph node cells, LNC, display distinctly different responses to BP in culture. The ability of both populations of cells to transfer EAE is enhanced after culture with antigen but the LNC require an additional signal in culture. This difference is being exploited to identify the subpopulations of T cells responsible for EAE induction and the role they play in regulation of this autoimmune cell mediated disease.

PHS-6040
(Rev. 10-76)

Project Description:

EAE is induced in susceptible animals by injection with either whole CNS tissue or purified myelin basic protein (BP) in complete Freund's adjuvant (CFA). The subsequent immune response leading to the development of clinical and histologic disease is T cell mediated and is assumed to be a delayed type hypersensitivity reaction since animals develop positive skin reactions when injected with BP and their cells both proliferate and produce lymphokines upon contact with BP in vitro.

We are currently investigating the role of various antigenic determinants on the BP molecule in disease induction by analyzing their interactions with various immune T cell subpopulations. This work was made possible by our previously reported system for enhanced adoptive transfer of EAE. We observed that as few as 10^7 PEC were capable of transferring full-blown EAE after 24 hr culture with BP. PEC from BP-sensitized donors also proliferate in the presence of BP.

We then attempted similar experiments with lymph node cells (LNC) from sensitized guinea pigs. However, culture of LNC with antigen did not lead to enhanced transfer or proliferation. Addition to the culture of normal syngeneic (Strain 13) PEC as a source of accessory cells did not improve transfer. We discovered that, in contrast to PEC, LNC require an additional signal in culture besides the presence of antigen. This second signal is provided by incorporating allogeneic (Strain 2) cells in the culture. The presence of the allogeneic cells (which differ from Strain 13 cells at the I region of the guinea pig major histocompatibility complex) leads to intense proliferation. This proliferation alone is not sufficient to induce enhanced transfer - the presence of BP in culture is critical. After 24 hr culture with Strain 2 PEC and BP, 2.5×10^7 sensitized LNC can transfer EAE in contrast to about 1×10^9 LNC that have not been cultured.

The Strain 2 PEC provide stimulation over a wide concentration range (5-30%) but do not play a primary role in disease transfer since they can be treated with Mitomycin-C (25 μg/ml) before culture with no demonstrable effect on the success of transfer. In contrast, treatment of the sensitized LNC with very low doses of Mitomycin-C (5 μg/ml) either before or after culture completely inhibits transfer. The two signals must be presented simultaneously to the sensitized cells in culture since mixing of cells cultured with BP alone with cells cultured with Strain 2 PEC alone does not result in cells capable of enhanced transfer.

Finally, after culture (but not before) the LNC capable of disease transfer can be found exclusively in a low density fraction on BSA gradients. This fraction contains only a small percentage of the total LNC. This result not only demonstrates that the cells responsible for transfer are proliferating blast cells but also offers a technique for enriching this antigen specific subpopulation.

To explain this difference between LNC and PEC we have hypothesized that sensitized PEC contain antigen-specific helper T cells, which proliferate in the presence of BP and provide help for antigen-specific effector T cells (which are ultimately responsible for the CNS tissue damage), whereas LNC contain only effector cells. Because of the lack of helper cells, LNC effector T cells in culture require non-specific help (provided by the allogeneic reaction) in order to respond to the BP in culture and differentiate into the cells responsible for CNS damage.

Publications:

Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Enhanced transfer of experimental allergic encephalomyelitis with strain 13 guinea pig lymph node cells: requirement for culture with specific antigen and allogeneic peritoneal exudate cells. J. Immunol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00903-03 LCM

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Preparation and Identification of Peptides Derived From Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. Deibler	Chemist	LCM	NIMH
Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)

Janis Young, McLean Hospital, Boston, Massachusetts
L. F. Eng, Veterans Administration Hospital, Palo Alto, California

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myelin basic protein (BP) presents a unique opportunity for a detailed study of the relationship between structure and biological activity--it has been completely sequenced and there is an impressive backlog of information on its encephalitogenic activity in several species; its ability to induce and bind antibody and its ability to bind other biochemical compounds or structures, specifically membrane constituents.

In order to study the relationship between structure and activity, we have prepared several well-characterized fragments of the protein by controlled proteolytic digestion and have purified several synthetic peptides prepared for us by Dr. Janis Young, Space Sciences Laboratory, University of California, Berkeley. Our purpose is to test the ability of these peptides to replicate the biological activity of the protein. By using fragments from different regions of the protein one can "map" active sites and define the minimal structural requirements for activity.

Project Description:

Last year we reported bioassay data on the activity of partially purified synthetic peptides related to the guinea pig encephalitogenic site. These peptides were characterized by amino acid analyses, high voltage electrophoresis (HVE) and two of them - the nonapeptide (113-121) and the longest peptide of the group (111-128) were partially sequenced with Carboxypeptidase Y.

From the bioassay data we conclude: 1) The BP-nonapeptide has definite encephalitogenic activity but the disease induced is milder than that induced by whole BP, even when a 50-fold molar excess of peptide is injected. 2) Extending the nonapeptide either by 2 residues on the N-terminal end or by 7 residues on the C-terminal end does not enhance its activity. 3) However, if the additional residues in 2) are added simultaneously, the specific activity is increased almost to that of BP.

Diagrammatically this can be expressed as follows:

NH$_2$/- Nonapeptide*- -/COOH

Mild activity NH$_2$- -/- - - - - - - - -/COOH

NH$_2$/- - - - - - - - -/- - - - - - - COOH

Improved activity NH$_2$- -/- - - - - - - - -/- - - - - - - COOH

* Each hyphen represents an amino acid residue.

One explanation for the improved activity of the longest peptide is that the additional residues enable the nonapeptide to assume its "native" conformation more readily--i.e., the conformation of the nonapeptide in the whole BP molecule--and that this conformation is optimum for encephalitogenic activity. We hope to be able to correlate the in vivo (encephalitogenic) activity of these peptides with their ability to replace BP in its reaction with specifically sensitized cells in vitro.

Inasmuch as conformation may be of considerable significance in the activity of these peptides it is important that we also have native peptides of varying lengths available. The latter are obtained through proteolytic digestion of BP. The fragments of most interest in our study of EAE in guinea pigs are those derived from the large C-terminal peptic peptide, residues 89 to 169. We now have peptide 89-127 (which is encephalitogenic because it includes the intact nonapeptide, residues 113-121) and peptides 89-119, 120-169, and 128-169, all of which are nonencephalitogenic. These fragments were obtained by digestion of BP with V-8 protease, an enzyme from Staphylococcus aureus.

In characterizing the V-8 peptides we used a relatively new carboxypeptidase (CPY) which was capable of hydrolyzing more C-terminal amino acids than CPA and CPB. CPY is known to be contaminated with an endopeptidase which may not be a problem with many globular proteins. Unfortunately, BP and its peptides are very susceptible to this endopeptidase, so much so that we were able to determine the specificity of the bonds split by the contaminating enzyme and to refute an earlier report on the ability of pepstatin to inhibit its activity. This study which was an outgrowth of our attempts to analyze the C-terminal residues of some newly isolated BP-fragments, resulted in some important new information about the use of CPY for C-terminal analysis and the specificity of its endopeptidase contaminant.

Publications:

Seil, F. J., Kies, M. W., Agrawal, H. C., Quarles, R. H., and Brady, R. O.: Myelin proteins dissociated from induction of antimyelin antibodies. In Giacobini, E., Vernadakis, A., and Shahar, A. (Eds). Tissue Culture Neurobiology. New York, Raven Press, 1980, pp. 477-488.

DeArmond, S., Deibler, G. E., Bacon, M. L., Kies, M. W., and Eng, L. F.: A correlated neurochemical and immunocytochemical study of P2 protein in the human and bovine nervous systems. J. Histochem. Cytochem. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00931-07 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies on the Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
	P. K. Chiang	Research Biologist	LGCB NIMH
Other:	Y. S. Im	Visiting Fellow	LGCB NIMH
	A. B. Guranowski	Visiting Associate	LGCB NIMH
	G. L. Kunz	Staff Fellow	LGCB NIMH
	R. R. Aksamit	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)

E. J. Leonard, Laboratory of Immunobiology, NCI
E. Schiffmann, Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2-5/6	5/6	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

After screening a large number of chemicals that are either analogs of adenosine or analogs of S-adenosyl-L-homocysteine (AdoHcy) that are modified in the base, ribose, sulfur or the homocysteinyl moiety, two most promising inhibitors of AdoHcy hydrolase have been found. They are 3-deazaadenosine (deaza-Ado) and 5'-deoxy-5'-(isobutylthio)-3-deaza-adenosine (deaza-SIBA). Deaza-Ado is unique in being both a substrate and also inhibitor of AdoHcy hydrolase. Deaza-Ado and other purine analogs, such as Ara-A, are also irreversible inhibitors of AdoHcy hydrolase. Both deaza-Ado and deaza-SIBA exert a broad spectrum antiviral activity against RNA and DNA viruses, as well as being able to prevent cellular transformation induced by oncogenic viruses. Both chemicals exhibit antimalarial activity against *Plasmodium falciparum* in culture. Deaza-Ado is a potent inhibitor of chemotaxis by neutrophil leukocytes, phagocytic function by macrophages and IgE-initiated histamine release from human basophils. When administered *in vivo* to rats, deaza-Ado caused a marked inhibition in the methylation of liver phospholipids, with concomitant reduction in the urinary excretion of 3-methoxy-4-hydroxy-mandelic acid and creatine biosynthesis in the liver.

Project Description:

Adenosylhomocysteine (AdoHcy) is the product of biological methylation reactions that utilize adenosylmethionine (AdoMet) as the methyl donor. As far as is known, the main pathway for the metabolism of AdoHcy in eukaryotes is its hydrolysis to adenosine and L-homocysteine by an enzyme, AdoHcy hydrolase. The reaction is reversible, with equilibrium far in the direction of synthesis:

$$\text{AdoHcy} \underset{H_2O}{\overset{\text{AdoHcy hydrolase}}{\rightleftharpoons}} \text{Adenosine} + \text{L-Homocysteine}$$

Many biological substances are methylated by AdoMet, such as biologenic amines, proteins, amino acids, lipids, carbohydrates, histones, myosine, DNA, and RNA. Interestingly as a rule, almost all of the enzyme methylases involved are sensitive to product inhibition by AdoHcy.

We focused our attention on AdoHcy per se as a biological modulator of methylation reactions, and many analogs of AdoHcy have been synthesized, and their efficacies as inhibitors of biological methylations studied. We are combining a study of the molecular properties of AdoHcy hydrolase and its regulation by effectors, with a detailed pharmacological exploration of its perturbation *in vivo* and *in vitro* by potent inhibitors, e.g. 3-deazaadenosine and 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine. The biological consequences of such a perturbation may be of paramount medical importance as well as yielding knowledge on the biochemical interplay between AdoHcy and AdoMet.

AdoHcy hydrolase was successfully prepared by us from beef liver by crystallization. It was found that AdoHcy hydrolase can be irreversibly inactivated by a large number of purine analogs. The irreversible inhibition of AdoHcy hydrolase by some purines could be due to a suicidal mechanism. Among the more potent irreversible inhibitors are 2-chloroadenosine, Ara-A, and (±)aristeromycin, in that order. After incubation with [^{14}C]adenosine and [^{3}H]Ara-A, tight binding of these ligands onto the enzyme can be demonstrated by gel electrophoresis. The enzyme activity lost after incubation with these purine analogs cannot be restored even after electrophoresis. Irreversible inactivation of AdoHcy by some purine analogs is probably a result of a suicidal mechanism, as suggested by our observations that adenine is cleaved from adenosine and Ara-A and 3-deazaadenine from 3-deazaadenosine by AdoHcy hydrolase. However, this suicidal inactivation of AdoHcy hydrolase by Ara-A can be completely prevented by the substrate, AdoHcy itself. The release of adenine from Ara-A or adenosine can be demonstrated when incubated with AdoHcy hydrolase. It was also found that eleven nucleosides can substitute for adenosine to generate their corresponding AdoHcy congeners: 3-deazaadenosine, 2-ara-3-deazaadenosine, nebularine, formycin, N^{6}-methyladenosine, 8-azaadenosine, adenosine-N^{1}-oxide, pyrazomycin, 8-aminoadenosine, inosine and (±)aristeromycin.

An exciting aspect of the utility of AdoHcy analogs begins to develop in the field of neuroscience. In collaboration with Dr. Marshall Nirenberg and his co-workers, it was found that both 3-deazaadenosine (DZA) and 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine (deaza-SIBA) exert profound inhibition of synaptic muscle responses in tissue culture. The tissue culture system consists of neurons from 8-day chick embryo retina grown in culture with rat striated muscle cells for 24 hours to form synapses. After treating the cultures for 2 hours with either analog, the number of muscle responses/min in these synapses was inhibited up to 95% by deaza-SIBA (I_{50} = 3 x 10^{-5} M), and DZA (I_{50} = 1.5 x 10^{-4} M). Homocysteine thiolactone, 5'-deoxyadenosine and tubercidin, which do not increase levels of AdoHcy or inhibit methyltransferases, had no effect. Deaza-SIBA inhibited the muscle responses with a half-time of 3.5 min. No inhibition of the number of synapses formed was seen. Inhibition of the number of muscle responses/min by DZA was reversed within 2 hours after removal of the compound. Furthermore, homocysteine thiolactone potentiated the inhibition of DZA by 6-fold when the two compounds were added together. These results strongly suggest that a methyltransferase-meadiated reaction occurs during neurotransmission, and also point to research possibilities in neuroscience quite exciting to come.

We continue to study the effects of DZA, and deaza-SIBA as antiviral agents in tissue culture systems. Both DZA, and deaza-SIBA exert a broad spectrum antiviral activity against RNA and DNA viruses: Rous sarcoma virus, Gross murine leukemia virus, Sindbis, Newcastle disease virus, vesicular stomatitis, influenza virus, Simian virus 40, herpes simplex, small pox, and HL-23 virus. Moreover, both DZA and deaza-SIBA can prevent cellular transformation induced by oncogenic viruses and DZA itself is able to reverse the malignant transformation induced by Rous sarcoma virus and to restore normal growth patterns.

An interesting facet of the usefulness of DZA and deaza-SIBA is their ability to elucidate immunological responses by leukocytes. It is postulated that cell (chemotaxis) migration toward a gradient response involves methylations of the cell membranes. DZA is the most potent purine analog that can inhibit chemotaxis by neutrophil leukocytes, with an I_{50} of 7 x 10^{-6} M. Other analogs, such as ^{6}N-methyladenosine, purine riboside, and deaza-SIBA can also inhibit chemotaxis. Another aspect of cellular motility is phagocytosis by macrophages. Again, DZA can inhibit phagocytosis as low as 1 μM. Additionally, DZA can inhibit IgE-initiated histamine release from human basophils with an IC_{50} of ∿1 mM. This inhibition was potentiated by L-homocysteine thiolactone. The inhibition by DZA was not potentiated by two different phosphodiesterase inhibitors suggesting that the action of DZA is not through changes in intracellular levels of cyclic AMP. DZA inhibits during the Ca^{++}-independent activation step of IgE-mediated basophil histamine release. In contrast, when histamine release was induced by the calcium ionophore A23187, fMet-Leu-Phe or zymosan-activated serum there was neither inhibition or enhancement of histamine release by these inhibitors of transmethylation. These results suggest that AdoMet-mediated methylation plays an important role in IgE-initiated histamine secretion from human basophils, whereas release induced by A23187, fMet-Leu-Phe or C5a bypasses this reaction step.

Our results taken together, suggest that DZA can perturb methylation reactions of cell membranes, by altering the AdoMet/AdoHcy ratio. This hypothesis is borne out by the findings that AdoHcy is drastically increased in these cells along with the appearance of 3-deaza-AdoHcy after incubation with DZA.

The potential use of DZA and deaza-SIBA as biochemical as well as pharmacological probes of cellular functions is unlimited and will remain quite illuminating yet.

Publications:

Chiang, P.K., and Cantoni, G.L.: Perturbation of biochemical transmethylations by 3-deazaadenosine *in vivo*. Biochem. Pharmacol. 28: 1897-1902, 1979.

Chiang, P.K., Im, Y.S., and Cantoni, G.L.: Phospholipids biosynthesis by methylation and choline incorporation: effect of 3-deazaadenosine. Biochem. Biophys. Res. Commun. 94: 174-181, 1980.

Cantoni, G.L., and Chiang, P.K.: The role of *S*-adenosylhomocysteine hydrolase in the control of biological methylations. In Cavallini, D., Gaull, G.E., and Zappia, V. (Eds.): Natural Sulfur Compounds. New York, Plenum Press, 1980, pp. 67-80.

Morita, Y., Chiang, P.K., and Siraganian, R.P.: Effect of inhibitors of transmethylation on histamine release from human basophils. Biochem. Pharmacol., in press.

Trager, W., Tershakovec, M., Chiang, P.K., and Cantoni, G.L.: *Plasmodium falciparum* in culture: antimalarial activity of sinefungin and other methylation inhibitors. Parasitology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00932-08 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Muscle Differentiation in Tissue Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: B. W. Uhlendorf Res. Microbiologist LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
½	½	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myogenesis *in vitro* has been much studied as a model of cellular differentiation. The formation of multinucleated non-proliferating electrically active and contractile myotubes by fusion of undifferentiated, proliferating myoblasts is accompanied by the appearance of muscle specific proteins, such as creatine kinase and myosin, and by the arrest of DNA synthesis. By manipulating the environmental conditions of a cloned line of myoblasts, I have been able to study these morphological and biochemical events. In addition, I have ellucidated the nature of one of the cues that signal the cell to proceed along the path of differentiation rather than the path of continued proliferation, namely the concentration of the serum component of the nutrient medium.

PHS-6040
(Rev. 10-76)

Projection Description:

Incubation in medium containing a low concentration of serum (1%) allows the cells to undergo certain covert changes which result in terminal differentiation, while incubation in a high concentration of serum does not permit these changes to occur, instead the cells continue to proliferate. The nature of the covert changes is not known. This project has been terminated.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00933-06 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study of Adenosylmethionine Synthetase and Enzymes of Transmethylations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
	P. K. Chiang	Research Biologist	LGCB NIMH
Other:	Y. S. Im	Visiting Fellow	LGCB NIMH
	A. B. Guranowski	Visiting Associate	LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Molecular properties of AdoMet synthetase and enzymes of transmethylation are being characterized. Guanidoacetate methyltransferase has been purified. Guanidoacetate methyltransferase has been purified about 140-fold from pig liver. Polyacrylamide gel electrophoresis of the purified enzyme showed four protein bands, each of which is associated with guanidoacetate methyltransferase activity. During gel electrophoresis at pH 3 in 8 M urea, guanidoacetate methyltransferase migrated as a single component. The molecular weight of the purified guanidoacetate methyltransferase was estimated to be 31,000 by sodium dodecyl sulfate-gel electrophoresis, which also showed only one protein component with guanidoacetate methyltransferase activity. Guanidoacetate methyltransferase is inhibited by adenosylhomocysteine, 3-deazaadenosylhomocysteine, and sinefungin with K_i values of 16 μM, 39 μM, and 18 μM, respectively.

PHS-6040
(Rev. 10-76)

Project Description:

Adenosylmethionine (AdoMet) a product of ATP plus methionine formed by the enzyme AdoMet synthetase, is one of the most versatile compounds in biology. It can function as a methyl donor in a very large number of transmethylation reactions, such as the methylation of biogenic amines, lipids, DNA, RNA, peptides myosin, amino acids, membranes, and myelin. Depending on the particular reaction or product involved, a biological reaction can either be initiated, activated or terminated after the transmethylation reaction. It can also function as a donor of a 3-amino-3-carboxypropyl group, of the amino group of homocysteinyl moiety, and after decarboxylation, of a propylamine side chain. In addition, it is cleaved enzymatically to generate thiomethyladenosine and homoserine lactone. Moreover, it can function as an allosteric effector in a variety of reactions.

For many years, there has been considerable interest in the biochemistry and pharmacology of biological transmethylation in the field of neuropsychiatry, which has been largely due to the fact that many natural or synthetic substances produce a variety of psychotropic effect, including hallucinations or other reactions that also occur in psychotic illness, are methylated amines. It has been suggested in the early fifties that abnormal transmethylation of an endogenous amine, possibly dopamine, might produce a psychomimetic compound like mescaline. Circumstantial evidence consistent with this hypothesis was the observation that methionine, when combined with an inhibitor of monoamine oxidase, could lead to striking and reversible exacerbations of the psychotic symptoms of chronic schizophrenia patients without inducing signs of delirium. There have been reports that AdoMet levels in the blood of acutely psychotic, but not chronically psychotic, schizophrenic subjects are low, indicating possibly either an excessive utilization or deficient production of AdoMet. An interesting study recently reported that intravenously injected AdoMet could exert clinically useful psychotic effects in depression. Moreover, initial findings of altered methionine-methyl metabolism in the leukocytes of schizophrenic patients have been reported, suggesting an abnormality in transmethylation process and/or an alteration in the metabolic pathway for the oxidation of this methyl group to CO_2. The fact that this abnormality was demonstrated in leukocytes suggests that the metabolic disorder associated with schizophrenia may be manifested in the general metabolism of the body. If these preliminary findings are confirmed by further studies, they not only will support the hypothesis of a transmethylation defect as a biochemical basis for schizophrenia, but also suggest a new approach to the study of this disease.

In view of the undisputed importance of AdoMet in transmethylation, Dr. Chiang continues to study the enzyme AdoMet synthetase, which produces AdoMet. More potent inhibitory analogs of methionine are still explored. The important discovery by Dr. Chiang of the two forms of AdoMet synthetase has now been confirmed by a research team in France, and by others for the enzyme from liver.

Since the demand for the labile methyl groups from the methionine source is determined largely by the requirement for creatine synthesis and the enzyme responsible for the synthesis of creatine, guanidoacetate methyltransferase

(GAA-MT), has received little attention in spite of its importance in methyl balance, we carried out the purification and detailed study of GAA-MT.

GAA-MT has been purified 140-fold from pig liver. Polyacrylamide gel electrophoresis of the purified enzyme showed from protein bands, each of which is associated with GAA-MT activity. When subjected to gel electrophoresis at pH 3 in 8 M urea, GAA-MT migrated as a single component. The molecular weight of the purified GAA-MT was estimated to be 31,000 by sodium dodecyl sulfate gel electrophoresis, which also showed only one protein component with GAA-MT activity. This molecular weight is in agreement with that estimated by Sephadex G-75 chromatography. GAA-MT is inhibited by adenosylhomocysteine, 3-deazaadenosylhomocysteine, and sinefungin with K_i values of 16 μM, 39 μM and 18 μM, respectively, whereas the K_m for AdoMet was 49 μM.

The strong inhibitory potency of adenosylhomocysteine and 3-deazaadenosylhomocysteine on the enzyme GAA-MT *in vitro* is thus constant with the observation that after the administration of 3-deazaadenosine in rats resulted in about 50% reduction in the level of creatine in rat livers. The reduction in the creatine level was assumed to be due to inhibition of creatine biosynthesis in the liver as a result of the increased intracellular concentration of Ado-Hcy, the formation of a large amount of 3-deaza-AdoHcy, and the accompanying changes in the ratio of AdoMet/AdoHcy or more specifically, AdoMet/(AdoHcy + 3-deaza-AdoHcy).

Chemotaxis by neutrophils is a tactile movement of a population of leukocytes towards a target, and normally the target is an invading microorganism to the host. The neutrophils respond to a chemical stimulus gradient towards the target. During the sensory transduction process, the membranes of the neutrophils undergo busy and intense methylation and demethylation processes. The methylation and demethylation processes can either act as signals or as results of cell motility. By the use of 3-deazaadenosine, which inhibits the adenosylhomocysteine hydrolase to produce an accumulation of adenosylhomocysteine (a methylation inhibitor), neutrophil chemotaxis could be blocked. The inhibition in chemotaxis can be correlated with blocks in transmethylation processes. The physiological roles played by transmethylation in cell motility will be quite exciting to come.

Publications:

Schiffman, E., O'Dea, R.F., Chiang, P.K., Venikatasubramanian, K., Corcoran, B., Hirata, F., and Axelrod, J.: Role for methylation in leukocyte chemotaxis. In Atkinson, D.E., and Fox, C.F. (Eds.): Modulation of Protein Function. New York, Academic Press, 1979, pp. 299-313.

Im, Y.S., Chiang, P.K., and Cantoni, G.L.: Guanidoacetate methyltransferase: purification and molecular properties. J. Biol. Chem. 254: 11047-11050, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00934-08 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies on the Biochemical Basis of Narcotic Drug Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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Laboratory of Neurosciences, NIA

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate receptors were, for the first time, extracted from membranes in an active and soluble state. Solubilization was effected both from brain and neuroblastoma x glioma NG108-15 hybrid cell membranes. The solubilized receptors are large complexes with stokes radii of 70 Å, and contain protein as an essential component. A new class of detergent, CHAPS, a zwitterionic (sulfobetaine) derivative of cholate was employed in these studies.

Membranes from NG108-15 cells have continued to be useful for study of the dual regulation of adenylate cyclase by opiates and opioid peptides in cell-free systems. The purified membrane system appears to require the only addition of opiates, ATP and, probably, GTP. This is the simplest biochemical model of opiate tolerance and dependence developed to date.

Project Description:

A necessary first step in the detailed study of the structure and mechanism of action of opiate receptors is their separation, in an active form, from membranes. The many attempts to solubilize active opiate receptors made in the past were uniformly unsuccessful. Preformed complexes of opiate with receptor had been solubilized, but the presumed receptor material in solution could no longer reversibly bind opiates. We have recently achieved success in the solubilization of opiate receptors in a state that reversibly binds opiate ligands. A new type of detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), a zwitterionic derivative of cholic acid, is used for solubilization of active opiate receptors from the membranes of neuroblastoma x glioma hybrid, NG108-15, cells as well as of rat and beef brain. Optimum solubilization conditions, opiate binding characteristics and hydrodynamic properties are similar for the receptors prepared from the three sources.

The solubilized receptors are large complexes, with molecular weights of the order of 300,000. Conditions which would be expected to dissociate the receptor into smaller species result in the loss of receptor activity. Protein is an essential component of this complex, since binding activity is lost after treatment with trypsin or alkylation with N-ethylmaleimide. Since the presence of detergent is necessary for binding activity the opiate receptor is apparently an integral membrane protein.

The solubilized receptors are present at approximately 1 pmol per mg of soluble protein. Purification of functional receptors is thus an attainable goal. The purification of active receptors will furnish the means to directly answer the question of the number of different types of opiate receptors in brain and other tissues. Although opiate receptors are normally coupled to adenylate cyclase in NG108-15 cell membranes, the solubilized receptors are uncoupled from the enzyme. Opportunities are thus created to study the mechanism of opiate receptor-adenylate cyclase coupling by suitable reconstitution experiments with purified, soluble receptors, and purified, soluble enzyme. Such studies would be expected to shed further light on the mechanism of acute opiate actions as well as on the phenomena of tolerance and dependence.

In our studies of the mechanism of opiate action, we have continued to exploit the favorable properties of the neuroblastoma x glioma cell line, NG108-15. This hybrid cell has a large number of opiate receptors which are functionally coupled to the enzyme, adenylate cyclase, as inhibitors. The cells become tolerant to and dependent upon opioids as a result of being cultured in their presence for 12 hours or longer. This addictive process is a homeostatic mechanism which serves to maintain cellular cyclic AMP levels near their normal values. The acute effects of opiates are to inhibit adenylate cyclase and thereby rapidly lower cAMP levels; the chronic adaptation is due to a gradual expansion in the amount of enzyme activity which serves to maintain normal cAMP levels even in the continued presence of morphine. The increased enzyme activity is due, at least in part, to changes in the regulatory properties of the pre-existing enzyme rather than to an increase in the number of enzyme molecules in the cell. We have developed a cell-free preparation which acquires the biochemical attributes of opioid tolerance and dependence. Membranes, prepared from neuroblastoma x glioma hybrid NG108-15 cells, contain functional opioid receptors which remain coupled, as inhibitors to adenylate cyclase. Incubation of these membranes with opioids for 30-60 minutes in the presence of ATP and GTP leads to the compensatory increase in adenylate cyclase activity. The membranes thus become tolerant to the inhibitory action of opioids and exhibit dependence as well, since a naloxone challenge allows the expression of a supernormal amount of enzyme activity. Because of the ease of manipulation of the cell-free system, it is possible to examine features of the mechanism of acquisition of opioid tolerance and dependence in molecular detail. Induction of dependence in this cell-free system requires that more than half of the opioid receptors be occupied with opioid agonists. Thus, mixed agonists such as cyclazocine, nalorphine and buprenorphine do not lead to dependence in this system, in agreement with results obtained with animals. It has been found that the degree of dependence induction in the membranes is closely correlated to the agonist efficacy of a series of opioids. Thus this system provides a simple, rapid and quantitative test for physical dependence liability of opioids. The direct quantitation of dependence liability has not heretofore been possible.

The opioid peptides behave in qualitatively identical fashion in the neuroblastoma x glioma hybrid cell and cell membrane systems as do the alkaloid opiates such as morphine. Thus, tolerance and dependence can be observed in whole cells and in cell-free systems with the opioid peptides methionine enkephalin and β-endorphin. Indeed, enkephalin shares with the alkaloid, etorphine, the property of being the most efficacious inducer of opioid dependence which we have found.

Our interest in opioid peptides has led us to search for, and find, enzyme activities in brain which may play a role in the biosynthesis of enkephalin from larger precursors. We have developed an amphiphilic substrate, based upon the peptide β-lipotropin (61-69) which is tagged with ^{14}C by conversion of lysine 69 to ^{14}C-homoarginine. The radioactive substrate is quantitatively absorbed to dextran-coated charcoal whereas the radioactive tetrapeptide product of enkephalin production remains in solution and can easily be measured. With this assay, two enzyme activities, one soluble and the other particulate, have been found in brain which produce enkephalin. The specificity of the cleavage was confirmed by chemical analyses. Based upon regional distribution and kinetic considerations, we believe that the particulate activity may well play a role in enkephalin biosynthesis whereas the soluble activity may function as a degradative enzyme. The particulate enzyme has been purified several hundred-fold by a series of chromatographic procedures. The purified enzyme is able to generate enkephalin both from β-lipotropin derived precursors and from, the newly discovered, α-neoendorphin. Large peptides such as β-endorphin are relatively poor substrates. The enzyme may therefore function as the catalyst of the last step in enkephalin biosynthesis. Hydrophobic bands in other peptides of moderate size are also cleaved by the enzyme so that its function may be a more general role in neuropeptide biosynthesis or breakdown.

Publications:

Klee, W.A.: Dual regulation of adenylate cyclase: a biochemical model for opiate tolerance and dependence. In Fishman, J. (Ed.): The Bases of Addiction. Berlin, Dahlem Workshops, 1978, pp. 431-440.

Zioudrou, C., and Klee, W.A.: Possible roles of peptides derived from food proteins in brain function. In Wurtman, R.J., and Wurtman, J.J. (Eds.): Nutrition and the Brain. Vol. 4, New York, Raven Press, 1979, pp. 125-158.

Klee, W.A.: Opioid peptides as modulators of cyclic AMP levels. In Ehrlich, Y.H., Volavka, J., Davis, L.G., and Brunngraber, E.G. (Eds.): Modulators, Mediators and Specifiers in Brain Function. New York, Plenum Press, 1979, pp. 225-231.

Knight, M., and Klee, W.A.: Enkephalin generating activity of rat brain endopeptidases. J. Biol. Chem. 254: 10426-10430, 1979.

Klee, W.A.: Peptides of the central nervous system. Adv. Protein Chem. 33: 243-286, 1979.

Bunney, W.E., Pert, C.B., Klee, W., Costa, E., Pert, A., and Davis, G.C.: Basic and clinical studies of endorphins. Ann. Intern. Med. 91: 239-250, 1979.

Knight, M., and Klee, W.A.: Purification of the enkephalin generating endopeptidase of rat brain membranes. In Gross, E., and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function. Rockford, Ill., Pierce Chemical Co., 1979, pp. 885-888.

Klee, W.A., and Streaty, R.A.: Multiple effects of opioid-receptor interactions in a clonal cell line. In: The Role of Peptides in Neuronal Function, 1979, in press.

Simonds, W.F., Koski, G., Streaty, R.A., Hjelmeland, L.M., and Klee, W.A.: Solubilization of active opiate receptors. Proc. Natl. Acad. Sci. U. S. A., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00935-13 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effect of Small Viruses and Their Nucleic Acids on the Biochemistry of Living Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. H. Ebert	Chief, Section on Experimental Therapeutics	LCS	NIMH
	L. Lipkin	Head, Image Processing Unit	LP	NCI
	P. Lemkin	Computer Scientist	LP	NCI
	G. C. Salmiraghi	Associate Director for Research		NIAAA
	D. Goldman	Research Associate		NIAAA
	R. Nelson	Research Biologist		NIAAA
	J. Horst	Professor, Human Genetics, University of Ulm, West Germany		

COOPERATING UNITS (if any)

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Laboratory of Clinical Science, NIMH
Dept. of Human Genetics, University of Ulm, West Germany

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section of Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lambda virus induction of discoordinate gene expression of the galactose operon has been shown to be due to translational control. This study was performed in collaboration with Drs. Gottesman and S. Adhya. Two-dimensional electrophoretic techniques were used in this study to provide evidence that an aberrant form of epimerase was not made during discoordinate gene expression of the galactose operon. The accurate location of the *E. coli* galactose operon proteins in two-dimensional electrophoretograms further allows this system to be used to monitor transfection experiments utilizing the *E. coli* gal operon in mouse L cells.

Clinically the two-dimensional electrophoretic system is being used to study genetic diseases. A two-dimensional map of the proteins in normal human spinal fluid has been plotted and 26 polypeptides have been identified. Preliminary results with the Lesch-Nyhan syndrome has shown that a combination of the two-dimensional electrophoretic techniques and computer analysis permits the identification of patients from normal controls in a double blind study.

Further work on protein detection in polyacrylamide gels by silver staining has resulted in the development of a new procedure which is six times faster than the old method and uses one hundreth the amount of silver.

Project Description:

Studies of lambda virus induced discoordinate gene expression of the galactose operon, have revealed that this phenomena is due to translational control. Previous studies in collaboration with Drs. Gottesman and Adhya have demonstrated a thousand-fold induction of the galactose-kinase enzyme with little if any increase of UDP-galactose-epimerase activity. This result was puzzling since the epimerase gene is closer to the lambda "leftward promotor" than the kinase gene. By the use of deletion mutants and "DNA-RNA" hybridization techniques it was shown that the mRNA's for each of the genes of the galactose operon were made in equimolar amounts. Furthermore, use of purified enzymes from the galactose operon as markers in a two-dimensional electrophoretic system allowed for an exhaustive search for an abnormal forms of epimerase gene product. No abberrant polypeptides were observed. A translational control model of the discoordinate gene expression became attractive when Dr. Joan Steitz (Yale University) noted a symmetrical sequence in the promotor-operator region of the gal-operon. The ribosomal binding site for epimerase is directly adjacent to the gal-operator-promotor region and is part of the "region of symmetry". Formation of a mRNA containing the symmetrical sequence results in a "stem and loop" structure with the epimerase ribosomal binding site contained in the "stem". Such a structure would not facilitate ribosomal binding required for translation of the epimerase gene. This model of translational control for the phenomenon of discoordinate gene expression has been further supported by use of insertion elements placed into the operator-promotor sequence to disrupt the symmetry. Disruption of the region of symmetry results in induction of epimerase enzyme activity by the lambda leftward promotor and reduction of the discoordinate effect.

Location of the *E. coli* galactose enzymes in two-dimensional electrophoretograms has permitted us to use this system as a probe for for *E. coli* gal-operon gene expression in Eucaryotic cells. In collaboration with Dr. J. Horst (University of Ulm, West Germany) clones of mouse L cells have been isolated, are being screened for *E. coli* gene expression. These clones were "rescued" by transfecting cells which were thymidine kinase deficient with DNA from Herpes simplex virus containing the Herpes thymidine kinase gene plus lambda virus containing the *E. coli* gal operon. Use is also being made of plasmids containing both eukaryotic and prokaryotic genes. Lysed cells from the isolated clones are being monitored for gal operon gene expression with the two-dimensional electrophoresis system. This system serves both as a detection system for specific gene expression, and it can also serve to monitor for possible contamination of the experiments by prokaryotic organisms.

Clinical application of the two-dimensional electrophoretic systems has been facilitated by the initiation of an NIMH-NIAAA collaborative project. This project's main goal is to determine the feasability of utilizing two-dimensional electrophoresis as a sensitive screening tool for abnormal proteins associated with disease, genetic markers and subtle differences in normally occurring proteins.

The initial studies in this collaborative project involved the development of a new silver stain for two-dimensional electrophoretograms that increases the threshold of detection of protein by approximately one thousand-fold. This allowed the study of proteins in very small tissue samples, in fluids such as cerebrospinal fluid that have very low concentrations of protein, as well as trace proteins that may have considerable biological significance. The initial phase of the clinical research in this project has involved the establishment of normal electrophoretic patterns of clinically obtainable tissues and fluids, e.g. serum, urine, cerebrospinal fluid, cellular elements of the blood, and fibroblasts. The electrophoretic positions of known proteins in these patterns have been established. In some cases, such as human serum, a molecular map of two-dimensional electrophoresis has already been published. In other cases, particularly samples that utilize the unique properties of the silver stain, we are establishing our own normal standards. Because of our interest in the genetics and metabolism of the central nervous system, we have completed an analysis of two-dimensional electrophoresis of cerebrospinal fluid. We are currently investigating the effect of acute and chronic ethanol administration on cerebrospinal fluid polypeptides. We are also working collaboratively with the Image Processing Unit, NCI to develop computer programs to facilitate the analysis and comparison of two-dimensional electrophoresis images. This approach appears important in clinical research applications of two-dimensional electrophoresis to set objective criteria for abnormal position or amount of a particular protein.

A major question which must be answered, prior to a large scale screening program for polypeptide markers in alcoholism, is whether our system of electrophoresis coupled with computer analysis can detect the polypeptides associated with genetic abnormalities. To test this system in a clinical situation, we have begun a double-blind study with individuals with the Lesch-Nyhan syndrome. This genetic disease is caused by an enzymatic defect in a purine salvage pathway. We have purified human enzyme and antibody to the involved enzyme. This has allowed us to establish the position of the normal enzyme in our electrophoretograms. Since this disease is due to a known protein aberration, it should serve as a good test of our system. Samples have been obtained from three Lesch-Nyhan patients and numerous controls. The absence of the enzyme activity has been verified in these patients by enzymatic assay.

Continued work with the silver stain for proteins in electrophoretograms has permitted the development of a rapid relatively inexpensive silver stain. The original stain developed in collaboration with Dr. Switzer (NIMH) was an adaption of an empirically developed histological stain for use on polyacrylamide gels. Once it was realized that the stain is based on the selective reduction of ionic silver to metallic silver at the protein sites, a process analagous to the silver based photographic methods, it was possible to adapt chemical techniques developed in photography to highly simplify the stain procedure. We now have a procedure which requires only three highly stable solutions and requires only 30 minutes. The original stain required three hours.

Publications:

Switzer, R.C., III, Merril, C.R., and Shifrin, S.: A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. Anal. Biochem. 98: 231-237, 1979.

Merril, C.R., Switzer, R.C., and Van Keuren, M.L.: Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proc. Natl. Acad. Sci. U. S. A. 76: 4335-4339, 1979.

Lemkin, P., Merril, C., Lipkin, L., Van Keuren, M., Oretel, W., Shapiro, B., Wade, M., Schultz, M., and Smithe, E.: Software aids for the analysis of 2-D gel electrophoresis images. Computers Biomed. Res. 12: 517-544, 1979.

Goldman, D., Merril, C.R., and Ebert, M.H.: Two-dimensional gel electrophoresis of cerebrospinal fluid proteins. Clin. Chem., in press.

Horst, J., Stanbro, H., and Merril, C.R.: On procaryotic gene expression in eukarytic systems. Humangenetik, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00936-17 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Homocystinuria: Methionine Metabolism in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. Feinleib	Chief, Epidemiology Branch	HV	NHLBI

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4/12	4/12	0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A survey of the health status of parents and grandparents of patients with homocystinuria due to cystathionine synthase deficiency has been completed. The aim was to determine whether the mild homocyst(e)inemia present in heterozygotes for this recessively inherited condition are at increased risk for atherosclerotic diseases: heart attacks and strokes. The information upon these relatives was compared to comparable information upon relatives of patients with either impaired phenylalanine metabolism or new mutation achondroplastic dwarfism.

Age-stratified comparison of the incidence of heart attacks and/or strokes failed to reveal significant increases consistently present in relatives of homocystinurics. Life table analyses of cumulative incidence of fatal heart attacks as a function of age likewise revealed no striking differences. No more than 5% of heterozygotes will have a coronary attack by age 50. These results indicate that mild homocyst(e)inemia is not an important contributory factor to atherosclerotic disease in the general population and provide no support for current hypothesis which postulate such an association.

PHS-6040
(Rev. 10-76)

Project Description:

During the interval since the last annual report, most of our efforts on this project have been devoted to the epidemiological study which seeks to determine whether heterozygotes for cystathionine synthase deficiency are at increased risk for early coronary artery disease. The rationale for undertaking this study and the experimental design have been covered in previous annual reports. During the past year, data collection from the study group and both control groups has been completed. Follow-up information was also obtained. The final numbers of responding families were: homocystinuria group, 203; impaired phenylalanine metabolism group, 156; new-mutation achondroplastic dwarfism group, 180. These represent 78.0, 74.3, and 81.1% of those receiving questionnaires.

Analyses of the data have now also been completed. Age-stratified incidences of heart attacks for each group of relatives (fathers, mothers, grandfathers, and grandmothers) in the homocystinuria study group were compared to the corresponding incidences for the two control groups. No striking increases in heart attacks were found among relatives of homocystinuric patients. Further analysis by means of the life table approach permitted calculation of cumulative chances of having heart attacks for each relative, determination of the differences in incidences between corresponding relatives for each group, and calculation of confidence limits for these differences. Again, no significant increases were consistently detected among relatives of homocystinuric patients. The latter analysis also permitted comparison of incidences of fatal heart attacks as determined by the present questionnaire method with those based upon the Framingham experience. The fact that the results were in good agreement served to validate the reliability of the methods used in this study.

To gain some insight into the effect of mild homocyst(e)inemia in groups free of hypertension and diabetes, known alternative risk factors for coronary disease, each of the analyses described above was repeated after prior removal from each group of individuals known to have one or both of these conditions. The results were not markedly different from those forthcoming from the previous analyses.

To ascertain whether geographical factors played a major role in the results obtained, the heart attack probabilities for the members of the families from North America only were compared with the control groups. The results were generally similar to those for the total groups.

When probabilities of strokes were analyzed in a similar manner no striking differences were detected for either fathers, mothers, grandfathers or grandmothers of homocystinuric children as compared to either control group.

Together, these results fail to provide any indication that mild homocyst(e)inemia, such as in present in heterozygotes for cystathionine synthase deficiency, is an important contributory factor to atherosclerotic disease in the population at large. We regard this negative conclusion as a significant one since it bears upon some of the hypotheses which suggest such a connection and which are currently being advanced in both scientific and popular journals. Certainly, widespread dietary or nutritional manipulations based upon such hypotheses should be very carefully scrutinized before being undertaken.

This work has now been written up and submitted for publication.

Publications:

Mudd, S.H., Ebert, M.H., and Scriver, C.R.: Labile methyl group balances in the human: the role of sarcosine. Metabolism, in press.

Mudd, S.H., Diseases of sulphur metabolism: implications for the methionine-homocysteine cycle, and vitamin responsiveness. Ciba Foundation Symposium 72: Biology of Sulphur, Excerpta Medica, North-Holland, Elsevier, 1980, pp. 239-258.

Schulman, J.D., Mudd, S.H., Schneider, J.A., Sheetz, M., Spielberg, S.P., Boxer, L.A., Oliver, J., and Corash, L.M.: Inborn errors of glutathione and sulfur amino acid metabolism. Ann. Intern. Med., in press.

Mudd, S.H.: Vitamin-responsive genetic abnormalities. Adv. Nutr. Res., in press.

Schulman, J.D., Mudd, S.H., Shulman, N.R., and Landvater, L.: Pregnancy and thrombophlebitis in pyridoxine nonresponsive homocystinuria associated with cystathionine synthase deficiency. Blood, in press.

Cogan, D.G., Schulman, J.D., Porter, R.J., and Mudd, S.H.: Epileptiform eye movements with methylmalonic aciduria and homocystinuria. Case report. Am. J. Ophthalmol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00937-15 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Transsulfuration in Higher Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. H. Datko	Botanist	LGCB NIMH
	G. A. Thompson	Sr. Staff Fellow	LGCB NIMH
	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3-8/12	3-8/12	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

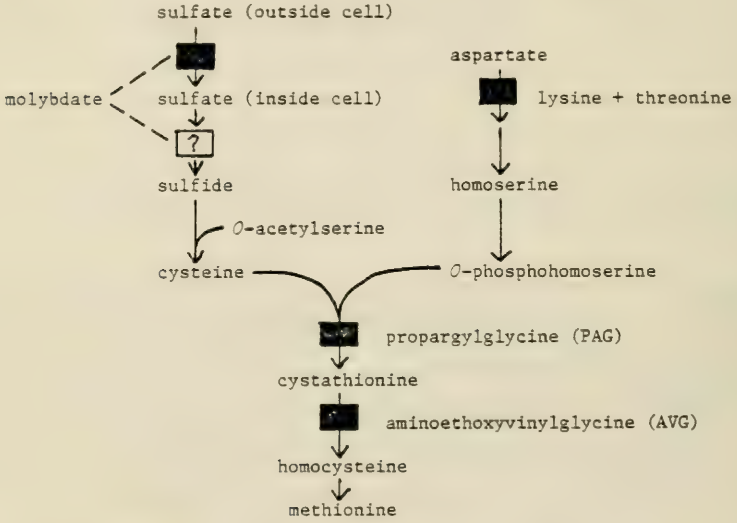
SUMMARY OF WORK (200 words or less - underline keywords)

Understanding of the regulation of methionine biosynthesis in *Lemna* was extended by the following results: a) a number of inhibitors which are specific for steps in various branches of the pathway were developed; b) the previous finding that exogenous methionine inhibits incorporation of sulfate sulfur into methionine was confirmed by an independent method but biological variability has for the moment prevented an unequivocal determination of whether it also inhibits the synthesis of its C4 moiety; c) methionine (or a derivative) non-allosterically regulates the amount and/or activity of cystathionine synthase. Growth in the presence of excess methionine decreases synthase activity 85%, whereas growth with limiting methionine increases activity 2- to 4-fold; d) the ratio of cystathionine synthase to *O*-phosphohomoserine (OPH) sulfhydrylase activity (1:1) remains constant under all growth conditions tested or when inhibited *in vitro* by propargylglycine. The ratio of *O*-acetylserine (OAS) sulfhydrylase to OPH sulfhydrylase activities (400:1 in control plants) varies over a 50-fold range under the same growth or inhibitor conditions. These data suggest that OPH sulfhydrylase activity is a property of cystathionine synthase whereas OAS sulfhydrylase is an activity of a separate enzyme.

Project Description:

A major goal of our research is to elucidate the mechanism of control of the biosynthesis of methionine and the other amino acids (lysine, threonine and isoleucine) of the aspartate family. We have previously validated the use of *Lemna* as an experimental material, and have continued to exploit the many advantages of this higher plant in the studies described below.

(1) A number of inhibitors were obtained which are specific for various branches of the methionine biosynthetic pathway:



The sites of inhibition by these compounds were elucidated by determining which intermediates are able to restore normal growth in the presence of each particular inhibitor. Thus growth inhibition by molybdate was relieved by cystine or excess sulfate (probably by competing with molybdate), but not by cystathionine, homocystine, or methionine. In addition to localizing the site of molybdate inhibition, these findings provide striking evidence that trans-sulfuration of homocysteine to cysteine is not of physiological significance in *Lemna*. Growth inhibition by lysine plus threonine was relieved by homoserine, cystathionine, homocystine or methionine, but not by sulfate or cystine. Propargylglycine (PAG) was first reported by other workers as an active-site-directed irreversible inhibitor of bacterial cystathionine γ-synthase. Our preliminary studies show that it acts similarly on this enzyme in

Lemna. Growth inhibition by PAG was relieved by methionine. Aminoethoxyvinylglycine (AVG) produced growth inhibition that was relieved by homocystine, or methionine, but not by sulfate or homoserine. Cystathionine relieved growth inhibition, probably by competing with the inhibitor for the active site of β-cystathionase. Further evidence for the sites of action of these inhibitors is now being obtained by determining the effects of the inhibitors on the accumulation of radioactivity from $^{35}SO_4{}^{2-}$ into intermediates of the pathway.

(2) In our previous studies to determine the physiological importance of feedback inhibition of methionine on its own synthesis, we found that addition of methionine caused a decreased *de novo* incorporation of sulfate sulfur into cystathionine and its products, but not into cysteine. This finding has been confirmed in a total of five experiments using two independent methods for determining *de novo* synthesis. Minimal values for *de novo* incorporation of sulfate sulfur in the presence of 2 μM methionine (expressed as a percentage of that with sulfate as the sole source of sulfur) varied from 22% to 47% (mean of 35%). Although the separate plant cultures used in each of the experiments were grown under identical conditions, biological variability probably accounts for the range in values.

While this finding is consistent with methionine feedback inhibiting its own synthesis at the cystathionine synthesis step, this interpretation becomes compelling only if it can be shown that *de novo* incorporation of the C4 and sulfur moieties are equally inhibited by exogenous methionine. We have made several attempts to provide evidence upon this question, but mainly because of biological variation between separate plant cultures, our present results do not allow a confident conclusion as to whether or not this requirement is satisfied. This problem is now being approached by assay of *de novo* incorporation of the C4 and sulfur moieties of methionine in the same plant culture by use of a double label (^{35}S and ^{14}C) technique.

(3) Studies on the *in vivo* incorporation of $^{35}SO_4{}^{2-}$ (see (2)) suggested that the cystathionine synthesis step could be a major site of regulation of methionine biosynthesis. It was therefore of interest to determine whether the activity of cystathionine synthase responded to growth conditions of methionine limitation or excess. Cystathionine synthase increased 2- to 4-fold after 72-96 hours growth in the presence of compounds (36 μM lysine plus 4 μM threonine, 0.05 μM AVG) that inhibit methionine biosynthesis, and decreased approximately 85% after two doublings in the presence of 2 μM methionine. Sulfate starvation had no effect on enzyme activity. These effects are not due to allosteric modifications of the enzyme, since enzyme activities were determined after gel filtration, and neither lysine and/or threonine, methionine, AdoMet or *S*-methylmethionine sulfonium at concentrations of at least 0.5 mM had any significant effect on enzyme activity. It is therefore concluded that methionine (or a derivative) regulated the amount and/or activity of cystathionine synthase by a mechanism other than allosterism. Non-allosteric regulation of enzyme activity is extremely rare in plants, and it is commonly believed that regulation by repression is unimportant in this phylum. It will therefore be of great interest to determine the molecular mechanism of the regulation of cystathionine synthase in *Lemna*.

(4) Since cystathionine synthase and OPH sulfhydrylase both catalyze a γ-elimination of phosphate from OPH coupled with attack by a sulfur-nucleophile e.g. cysteine, sulfide, or methyl mercaptan, it was possible that the two activities were properties of the same enzyme. The ratio of the two enzyme activities remained relatively constant over the 20-fold range in specific activities resulting from the various growth conditions described in (3). Furthermore, the activities of cystathionine synthase and the sulfhydrylase decreased rapidly and in parallel to 10% of the control activity either when *Lemna* was grown in the presence of PAG (20 mM) or when crude extracts were incubated with this inhibitor (30 µM) *in vitro*. These results suggest that the two activities are properties of the same enzyme (cystathionine-γ-synthase) and that in this regard *Lemna* more closely resembles bacteria rather than fungi. Purification of the enzyme will be required to confirm this suggestion.

(5) *O*-Acetylserine (OAS) sulfhydrylase activity might also be a property of OPH sulfhydrylase (cystathionine synthase) if it were possible for the OPH-dependent enzymes to utilize OAS in place of OPH. A number of lines of evidence indicate that this is not the case: a) The ratio of OAS-sulfhydrylase to OPH-sulfhydrylase activities (400:1 in control plants) did not remain constant under the growth conditions described in (3). Instead, the ratio varied from 200:1 when the plants were grown in the presence of lysine plus threonine to 2500:1 when *Lemna* was grown in the presence of exogenous methionine. b) OAS-Sulfhydrylase was not inactivated by PAG. c) Lanthionine was not produced when OAS and cysteine were incubated with enzyme extract.

Future work will be continued along the following lines:

(1) The ability to specifically block various steps of biosynthesis, together with the many advantages of the *Lemna* system (see previous annual reports) provide a unique opportunity for studies on the regulation of the enzymes for biosynthesis of methionine and other amino acids of the aspartate pathway. Already our studies have shown that the activity of cystathionine synthase is regulated in a non-allosteric fashion that is most unusual in plants, and it is planned to extend these studies to other relevant enzymes.

(2) The molecular mechanism for control of cystathionine synthase activity will be studied. As a first step, in collaboration with Dr. Merril's group, we plan to determine by two-dimensional gels to what extent the observed changes in enzyme activities could be due to changes in the amounts of the enzyme, or to its covalent modification.

(3) It is not clear why sulfate starvation had no significant effect on the activity of cystathionine synthase, whereas deprivation of methionine by other means (e.g. by inhibition by lysine plus threonine, AVG) caused marked increases. One explanation that will be examined is that increases in cystathionine synthase activity requires both negative and positive control, and that an adequate sulfate supply is required for synthesis of the positive effector.

(4) Plants are unique among all organisms studied in that cystathionine syn-

thase and threonine synthase are the first enzymes in the branch pathways to methionine and threonine, respectively. Present knowledge suggests that these enzymes probably play important regulatory roles. It would therefore be rewarding to purify these enzymes and study their properties in detail.

Publications:

Datko, A.H., Mudd, S.H., and Giovanelli, J.: *Lemna paucicostata* Hegelm 6746. Development of standardized growth conditions suitable for biochemical experimentation. Plant Physiol. 65: 906-912, 1980.

Datko, A.H., Mudd, S.H., and Giovanelli: *Lemna paucicostata* Hegelm 6746. Life cycle and characterization of the colony types in a population. Plant Physiol. 65: 913-923, 1980.

Giovanelli, J., Mudd, S.H., and Datko, A.H.: Homocysteine Biosynthesis in Plants. In Cavallini, D., Gaull, G. E., and Zappia, V. (Eds.): Natural Sulfur Compounds. New York, Plenum, 1980, pp. 81-92.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00938-01 LGCB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effect of B_{12} on Folate Uptake, Utilization and Interconversion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: B. W. Uhlendorf Res. Microbiologist LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: ½	PROFESSIONAL: ½	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(Rev. 10-76)

Project Description:

In the recent literature, there has been serious question as to whether the "methyl folate trap hypothesis" is sufficient to account for the megaloblastosis seen in B_{12}-deficiency. In fact, it has been suggested that the action of B_{12} here is to control the transport of folates into target cells.

In an attempt to document this, I am studying the uptake, utilization and interconversions of folates in normal human fibroblasts and in human fibroblasts having a defect in B_{12}-metabolism. I am using nitrous oxide to maintain the B_{12} in the coenzymatically inactive trivalent cobalt form. There are no experimental results as yet.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00981-15 LNB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Analysis of the Macromolecular Structure of Nerve Membrane During Excitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Tasaki	Chief	LNB NIMH
	S. Terakawa	Visiting Associate	LNB NIMH
	R. Gibbons	Electronic Tech.	LNB NIMH
	K. Iwasa	Senior Staff Fellow	LNB NIMH

COOPERATING UNITS (if any)

Marine Biological Laboratory
Woods Hole, Mass.

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of nerve excitation was studied from a macromolecular viewpoint. The birefringent signals associated with an action potential in a dye-loaded squid giant axon was studied and found to be much larger than that obtained from an intact axon. The mechanical changes associated with the production of action potentials were studied using three different methods. All three methods yielded consistent results indicating the occurrence of swelling of nerve fibers associated with an action potential. In addition to this swelling, nerve fibers were found to undergo a transient shortening in their length during an action potential. The magnitude of this shortening was found to be 5-10 nm and the tension developed, 15-20 μg. These findings suggest that cytoskeletal structures associated with the axolemma, are involved in the process of nerve excitation.

PHS-6040
(Rev. 10-76)

Publications:

Mautner, H.G., Lorenc, C., Quain, P., Marquis, J.K., and Tasaki, I.: Synthesis and study of conformationally defined enantiomers of local anesthetics and conformationally defined enantiomers of fluorescent dyes designed to label electrically excitable membranes. *J. Med. Chem*. 23: 282-285, 1980.

Pant, H.C., Pollard, H.B., Pappas, G.D., and Gainer, H.: Phosphorylation of specific, distinct proteins in synaptosomes and axons from squid nervous system. *Proc. Natl. Acad. Sci. U.S.A.* 76: 6071-6075, 1979.

Pant, H.C., and Gainer, H.: Properties of a calcium-activated protease in squid axoplasm which selectively degrades neurofilament proteins. *J. Neurobiol*. 11: 1-12, 1980.

Tasaki, I., and Iwasa, K.: Shortening of nerve fibers associated with propagated nerve impulse. *Biochem. Biophys. Res. Commun*., in press.

Tasaki, I., and Terakawa, S.: Oscillatory miniature responses in the squid giant axon: origin of rhythmical activities in the nerve membrane. In Carpenter, D.O. (Ed.): *Cellular Pacemakers*. New York, Wiley-Interscience, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00983-02 LNB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on the Mechanism of Nerve Excitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Baumgold	Senior Staff Fellow	LNB NIMH

COOPERATING UNITS (if any)

Marine Biological Laboratory
Woods Hole, Mass.

LAB/BRANCH
Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Proteins involved in nerve excitation were studied using two main approaches. One approach was aimed at studying the membrane-associated proteins. Using one- and two-dimensional polyacrylamide gel electrophoresis, it was found that these proteins are mostly actin, tubulin and neurofilament protein. Furthermore, these proteins were found to exist in a KF soluble and KF insoluble form, an observation consistent with the view that these proteins undergo a transient depolymerization during nerve excitation. In the other approach, probes for the excitable sites of nerve were used. These probes included a toxin from scorpion venom and saxitoxin, both of which were radioactively labelled. The scorpion toxin was used to study the developmental course of excitable sites in tissue cultured muscle cells. The saxitoxin was used in order to purify the excitable sites.

Publications:

Baumgold, J.: Calcium ion binding to lobster nerve membranes. Biochim. Biophys. Acta 558: 149-165, 1979.

Baumgold, J.: ^{3}H-Saxitoxin binding to nerve membranes: inhibition by phospholipase A_2 and by unsaturated fatty acids. J. Neurochem. 34: 327-334, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01031-12 LNC

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Conversion of Phenylalanine to Tyrosine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Sheldon Milstien	Research Chemist	LNC NIMH
	Michael Parniak	Visiting Fellow	LNC NIMH
	Lauraine Shaw-Goldstein	Staff Fellow	LNC NIMH
	Hiroyuki Hasegawa	Visiting Fellow	LNC NIMH
	Harvey Wilgus	Staff Fellow	LNC NIMH
	Berenice Gitomer	Visiting Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 7.7	PROFESSIONAL: 6.2	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methylenetetrahydrofolate reductase has dihydropteridine reductase activity. High concentrations of phenylalanine stimulate biopterin synthesis in rats.

Project Description:

The objective of this research project is the detailed description of the complex enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components of the hydroxylation system. These components include phenylalanine hydroxylase dihydropteridine reductase, and tetrahydrobiopterin.

A related objective is to use this basic knowledge of the phenylalanine hydroxylase system to increase our understanding of the disease, phenylketonuria, (PKU), as well as its variants. Specifically, the goal is to devise methods of detection and therapy for these diseases, as well as to understand their pathogenesis.

We have continued our studies of the regulation of hepatic phenylalanine hydroxylase by phosphorylation-dephosphorylation processes. A phosphatase that can catalyze the dephosphorylation of phenylalanine hydroxylase has been purified extensively from rat liver extracts. The loss of phosphate from the hydroxylase is accompanied by loss of tetrahydrobiopterin-dependent hydroxylase activity. We had previously found that phosphorylation of phenylalanine hydroxylase leads to a shift in its elution pattern from calcium phosphate columns. We have now found that dephosphorylation of phenylalanine hydroxylase by the phosphatase causes a shift in its elution pattern back to its original position. These results prove that *in vitro* there is a complete cycle of phosphorylation-dephosphorylation.

In collaboration with Dr. Rowena Matthews, University of Michigan, we have found that methylene tetrahydrofolate reductase has dihydropterin reductase activity. This enzyme can, therefore, support phenylalanine hydroxylation in the liver and tyrosine and tryptophan hydroxylation in the brain. This finding may explain why patients with a genetic deficiency of methylene tetrahydrofolate reductase also have defects in neurotransmitter synthesis. Deficiencies in neurotransmitters, in turn, would likely contribute to the neuropathology seen in these patients.

We have previously found evidence that indicates that the synthesis of biopterin in humans is stimulated by high levels of phenylalanine. Since biopterin is essential for the metabolism of phenylalanine, this effect of phenylalanine on biopterin synthesis could be an important regulatory mechanism. During the last year, we have shown that this effect of phenylalanine on biopterin synthesis can be duplicated in rats. In addition, we have demonstrated in rats that phenylalanine stimulates the conversion of radioactive guanosine, a known precursor of biopterin, to biopterin. These results show directly that phenylalanine can stimulate the synthesis of biopterin. With this animal model, we hope to characterize the system further and to identify the step in the biopterin biosynthetic pathway that is sensitive to phenylalanine levels.

Publications:

Milstien, S., Kaufman, S. and Summer, G.K.: Hyperphenylalaninemia due to dihydropteridine reductase deficiency: Diagnosis by measurement of oxidized and reduced pterins in urine. Pediatrics 65: 806-810, 1980.

Kaufman, S.: Differential diagnosis of variant forms of hyperphenylalaninemia. Pediatrics 65: 840-842, 1980.

Donlon, J. and Kaufman, S.: Relationship between the multiple forms of rat hepatic phenylalanine hydroxylase and degree of phosphorylation. J. Biol. Chem. 255: 2146-2152, 1980.

Matthews, R.G. and Kaufman, S.: Characterization of the dihydropterin reductase activity of pig liver methylenetetrahydrofolate reductase. J. Biol. Chem. in press.

Nixon, J.C., Lee, C.-L., Milstien, S., Kaufman, S. and Bartholome, K.: Neopterin and biopterin levels in patients with atypical forms of phenylketonuria. J. Neurochem. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01032-12 LNC

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biosynthesis of Catecholamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Gregory Kapatos	Staff Fellow	LNC NIMH
	Bruce Gomes	Staff Fellow	LNC NIMH
	Setsuko Katoh	Visiting Associate	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines, is dependent upon a reduced unconjugated pteridine, tetrahydrobiopterin. Utilizing rat brain and neuroblastoma preparations we have analyzed pteridine content and biosynthesis in order to elucidate the pathway by which these compounds are synthesized in neural tissues, with the eventual goal of understanding the cellular interactions between tyrosine hydroxylase and its pteridine cofactor.

PHS-6040
(Rev. 10-76)

Project Description:

Of the possible pteridines detectable by our methodology rat brain was found to contain 120 ng/g of biopterin (2-amino-4-hydroxy-6-[1,2-dihydroxypropyl-(L-erythro)]-pteridine) over 90% of which was tetrahydrobiopterin (BH_4). In contrast to the findings of others, repeated experimentation has demonstrated the difficulty of detecting radioactive BH_4 synthesized by rat brain following intraventricular injections of radiolabeled precursors. For example, four hours following an injection of 30 µCi of ^{14}C-guanosine only 300 C.P.M. can be detected in biopterin fractions. We have therefore attempted to analyze the biosynthetic pathway by supplying the brain with presumed intermediates of BH_4. One such compound is sepiapterin (SP; 2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine), which can be converted by brain in vitro to 7,8-dihydrobiopterin (BH_2) by the enzyme sepiapterin reductase. Intraventricular injection of SP increased brain BH_2 and BH_4 levels over seven-fold, suggesting that SP is normally an intermediate in BH_4 biosynthesis. However, later work demonstrated no correlation between sepiapterin reductase activity and BH_4 levels across various brain regions, or between the subcellular distributions of this enzyme and tyrosine hydroxylase. The role of SP and SP-reductase in BH_4 biosynthesis therefore remains equivocal.

Because of the difficulties encountered in radiolabel experiments with rat brain we have also utilized a tissue culture system. A sub-clone of the neuroblastoma N1E115 has been found to contain enormous quantities of BH_4 as well as D-neopterin (Neo; 2-amino-4-hydroxy-6-[1,2,3-trihydroxypropyl-(D-erythro)]-pteridine). Following determination of intracellular volume the concentrations of BH_4 and Neo were found to be 115 and 3 µM, respectively. In contrast to rat brain, incubation of these cells for one hour with labeled precursors results in incorporation of radioactivity into these pteridines to the extent of 100 D.P.M./ng. The addition of SP to the incubation medium inhibits incorporation of radioactivity into Neo BH_2, and BH_4 without altering the specific activity of the initial precursor guanosine triphosphate.

These neuroblastoma cells have also been used as a source of starting material for the purification of tyrosine hydroxylase. This enzyme has been purified more than 80-fold, and preliminary kinetic data indicate that the enzyme is in an activated conformation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01034-12 LNC

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Biochemical Basis of Skeletal Muscle Hypertrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Herman Vandenburgh	Sr. Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.25	PROFESSIONAL: 1.25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. Present topics of investigation include the relationship between stretch-induced hypertrophy and muscle protein synthesis, degradation, and changes in Na pump activity and cyclic nucleotide levels.

PHS-6040
(Rev. 10-76)

Project Description:

One hypothesis of the basis of skeletal muscle hypertrophy is that it is due to mechanical stretching of the muscle which leads to increased tension and increased work. This hypothesis has been confirmed using a technique developed in this laboratory of stretching monolayers of fowl embryonic skeletal myotubes differentiated in vitro. A continuous 10 percent stretch of the cells leads to increased amino acid accumulation, increased incorporation of amino acids into total cellular protein and muscle specific protein (myosin heavy chain), increased accumulation of total cellular protein and myosin heavy chain and decreased total cellular protein degradation. These changes are similar to those seen in in vivo models of skeletal muscle hypertrophy. Having established an in vitro model for skeletal muscle hypertrophy, we are currently testing several biochemical models for the stimulation of cell growth in this system. These models include changes in the activity of the sodium pump and in intracellular levels of cyclic nucleotides. Stretch of the skeletal myotubes has been found to activate the membrane Na pump (Na^+-K^+ ATPase). This Na pump activation appears to be essential for stretch-induced muscle cell growth since ouabain, a specific Na pump inhibitor, prevents the stretch-induced stimulation of amino acid transport and protein synthesis. Activation of the Na pump by stretch is due to an increase in the Vmax of the enzyme with little change in its Km. [^{3}H]ouabain binding studies indicate that there is no increase in the number of membrane Na pumps in response to stretch; therefore, the Vmax increase appears to be due to activation by stretch of the Na pumps already present in the cell membrane. We are currently exploring the possible mechanisms by which activation of the Na pump is coupled to skeletal muscle cell growth.

Publications:

Vandenburgh, H.H. and Kaufman, S.: Protein degradation in tissue-cultured skeletal muscle. Effect of medium, cell type, inhibitors, and mechanical stretch. J. Biol. Chem. in press.

Vandenburgh, H.H. and Kaufman, S.: In Vitro Skeletal Muscle Hypertrophy and Na Pump Activity. In Pette, D. (Ed.): Plasticity of Muscle. Berlin, Walter de Gruyter, 1980, pp. 493-506.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01035-12 LNC

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Process of Lysogeny

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Nash	Medical Officer (Research)	LNC NIMH
Other:	Thomas Pollock	Guest Worker	LNC NIMH
	Brenda Lange-Gustafson	Guest Worker	LNC NIMH
	Nancy Craig	Guest Worker	LNC NIMH
	Carol Robertson	Biologist	LNC NIMH

COOPERATING UNITS (if any) Division of Biology and Medicine, Brown University; Laboratory of Molecular Genetics, NICHD; Laboratory of Molecular Biology, NCI; Laboratory of Molecular Biology, NIAMDD

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To define the mechanism of integrative recombination the structure of the products of this reaction has been determined. These products are supertwisted circles of DNA that are linked to each other in a catenate. In addition, the pathway of relaxation of supertwisted DNA by a recombination protein has been resolved, this topoisomerase works by transient cleavage of single strands of DNA. A model for integrative recombination is proposed that accounts for these observations and for other features of the biochemistry of this reaction.

PHS-6040
(Rev. 10-76)

Project Description

The integration of viral DNA into host chromosomes is an important process that is distributed widely in nature. We have been studying the biochemistry of one such integration - the insertion of the DNA of bacteriophage lambda into the genome of its E. coli bacterial host. We first demonstrated that this recombination could be achieved in a cell-free system. Subsequently we identified and purified the proteins involved in this reaction and are now addressing basic questions of the mechanism of this genetic recombination.

One kind of mechanistic study is the characterization of the products of the recombination reaction. In early studies of integrative recombination we determined that supercoiled circular DNA is the primary substrate for this reaction and that some of the products are themselves supertwisted circles. We have now established that, starting with a supertwisted circle bearing two recombining sites, virtually all of the recombinants are circles. Moreover, each pair of circles arising from a single recombination event is catenated, i.e. the two circles are connected to each other like the links of a chain. Catenation might be a direct consequence of supertwisting of the circular substrate DNA. In such circles the interwinding of the double helix would frequently tangle segments of the substrate that are destined to reside in different product circles; these tangles would become the links of the catenate. We have tested this hypothesis by studying recombination of nonsupercoiled DNA, a reaction that proceeds only inefficiently and under restrictive ionic conditions. We find that the products of this unusual reaction are again circles of DNA but, in this case, at least some of these circles are unlinked to one another. Catenation is thus not an intrinsic feature of recombination but simply a consequence of the supertwisted nature of the normal substrate. The detailed structure of catenates arising from supercoiled substrates has also been informative. We frequently find that both partners in a single catenate are covalently closed circles. Thus, in the in vitro reaction as in viral integration in whole cells, all the ends created by the breakage step in recombination are rejoined. Taken together with the fact that recombination in vitro is performed in the absence of high energy cofactors like ATP, this means that hydrolysis of DNA plays no role in the cleavage step of recombination. All the ends must be made by a process that permits reversible rejoining. Most importantly, the observation that the circles in the catenates are not only closed but are also supertwisted proves for the first time that no significant swiveling of the broken ends can occur before the rejoining step of recombination.

The viral protein, Int, that catalyzes integrative recombination is functionally complex. This polypeptide displays specific binding to several sequences located at or near the sites of recombination. In addition, Int protein is a topoisomerase, i.e. it breaks and reseals DNA thereby converting supertwisted to relaxed circular DNA. We have asked whether this breaking and resealing occurs on a single strand of DNA, or involves, as in the case of DNA gyrase, double stranded breaks. By several criteria, Int is identified as of topoisomerase of the first type, i.e., a nicking closing enzyme. In an attempt to dissect some of the activities of Int and relate them to the overall recombination pathway, we are undertaking this study of mutant Int proteins. Temperature sensitive, chain terminating, and other alleles of the int gene have been cloned in a plasmid vector. The mutant proteins are being examined in crude and purified form for their interactions with DNA as well as with mutant and normal recombination proteins.

We have proposed a novel mechanism for recombination that incorporates the constraints defined by our studies of the products of recombination as well as our knowledge of the biochemical properties of the recombination proteins. The model invokes intimate juxtaposition of the recombining sites in a four-stranded helix prior to the cleavage of any DNA. This arrangement ensures that, after cleavage of DNA, minimal rotation of free ends will bring them into contact with new partners. This permits strand exchange to be catalyzed by a nicking closing enzyme and ensures that only minimal loss of superhelicity occurs during recombination. This mechanism accounts for these and other genetic and biochemical features of integrative recombination; naturally occurring genetic rearrangements in other species could also take place by such a mechanism.

Publications:

Kikuchi, Y. and Nash, H. A.: Nicking-closing activity associated with bacteriophage λ int gene product. Proc. Natl. Acad. Sci. 76:3760-3764, 1979.

Pollock, T. J. and Abremski, K.: DNA without supertwists can be an in vitro substrate for site-specific recombination of bacteriophage λ. J. Mol. Biol. 131: 651-654, 1979.

Ross, W., Landy, A., Kikuchi, Y. and Nash, H.A.: Interaction of int protein with specific sites on λ att DNA. Cell 18: 297-307, 1979.

Mizuuchi, K., Gellert, M., Weisberg, R. A. and Nash, H. A.: Catenation and supercoiling in the products of in vitro λ integrative recombination. J. Mol. Biol., in press.

Pollock, T. J. and Nash, H. A.: Catenation of The Products Of Integrative Recombination: Comparison of Supertwisted and Nonsupertwisted Substrates. In Alberts, B. and Fox, C. F. (Eds.): Mechanistic Studies of DNA Replication and Genetic Recombination - ICN-UCLA Symposia on Molecular and Cellular Biology, Volume XIX. New York, Academic Press, 1980, in press.

Nash, H. A., Mizuuchi, K., Enquist, L., and Weisberg, R. A.: Strand exchange in λ site specific recombination: Genetics, biochemistry and models. In Cold Spring Harbor Symposia on Quantitative Biology 45: 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01036-09 LNC

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Heparin-Induced Conformations: Model for Intracellular Polysaccharide-Protein Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Audrey L. Stone Research Chemist LNC NIMH

COOPERATING UNITS (if any)
Sidney Farber Cancer Institute, Harvard Medical School

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.35	PROFESSIONAL: 0.95	OTHER: 0.40

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Sequestering of cell solutes from soluble to organized structures was investigated with regard to conformations of interacting molecules. Glycosaminoglycans and acid polysaccharides were characterized by optical properties. The ordered, negative charges specifically ordered amine cations and exhibited conformation-promoting effects on nonordered cationic polypeptides. Heparin (and other polyanions) with poly L-lysine:L-tyrosine promoted β-structure, while with poly L-lysine: L-phenylalanine, α-helical order. That the nature of conformational change with heparin depended upon constellations of amino acids and their proclivity for α-helix or β-structures bears on the molecular basis of granule formation, enzyme and antienzyme activation. Antithrombin (AT) activation depends upon an unique binding site in 15% of heparin (H) chains, having greater specific activity at 20,000 d than 6000 d. Present structure-function results with H fractions revealed unique conformation of sugars in the AT binding sequence and enhanced secondary structure in large active chains. A molecular model for H regulation of tyrosine hydroxylase (TH) analogous with H-AT led to discovery of inhibition by cationic peptides which increased Kapp tyrosine. H produced high Ki forms in preparation of TH in *in vitro* assays.

Project Description:

Anionic polysaccharides (glycosaminoglycans (GAG)) are intimately involved in the biological formation and function of inter- and intra-cellular matrices and membranes and some, such as heparin, are known to stimulate or inhibit enzyme systems. Intracellular storage regions, or discrete granules, occur in various organs of many species, including synaptic processes of catecholaminergic neurons. Physicochemical forces which sequester cell solutes from a soluble form to a relatively insoluble, organized structure can be investigated from the standpoint of the molecular conformations of the components and of their interacting species, i.e., polysaccharides and proteins. Investigation of the macromolecular structure of anionic polysaccharides in solution was undertaken at the onset of this project in the early 60's. Results were the first to demonstrate ultraviolet asymmetric absorption bands for heparin and other GAG. Results with the cationic amine dye ligands led to the first reports of helical structures within these polysaccharides in solution. Both methods showed that GAG comprise two conformational classes in solutions near pH 7. The results also showed that ordered negative charges have specific ordering effects on amine cations. The project was extended to study interaction with polypeptides. As with small amines, acid polysaccharides (PS) played a role in inducing conformational constraints in cationic polypeptides, poly L-lysine:tyrosine (P(L:T)) and poly L-lysine:phenylalanine (P(L:P)). A major objective was to quantitatively determine the conformation-altering effect of various anionic PS, and to determine possible specificity of this effect among PS conformations. The current project applied these model interactions directly to studies of enzyme and antienzyme activation and sequestration by heparin and related biopolymers.

The work of Dr. Robert Rosenberg (at Sidney Farber Research Institute, HMS) had shown that the anticoagulant potency of heparin in the antithrombin system resides in chains comprising less than 10 percent of the bulk preparation. Furthermore, heparin interacted with at least two lysyl residues on antithrombin so as to complex with its tryptophan residue(s), change its conformation and enhance its inhibition of thrombin. Structure-function relations were revealed by our comparison of the ligand binding properties of the active chains with those of inactive heparin, and by elucidation of sugar conformations in the active, combining tetrasaccharide. The molecular basis of antithrombin activation was further defined by direct measurement of the optical properties of the heparin-antithrombin complex and by optical studies with the model polypeptides, P(L:T), P(L:P) and poly L-lysine:tryptophan. In collaboration with Dr. Robert Rosenberg, the optical properties of high molecular weight (20,000 d) and low molecular weight (6000 d), highly active and activity-depleted heparin chains were determined. Major findings showed a) that the polyanionic character of active and inactive chains were similar in many aspects but differed significantly in the development of stable regions of secondary order as a function of chain length, with the active chain exhibiting more stable secondary structure; b) the conformation of the sugar rings in the active tetrasaccharide combining site differed from that of the other repeating tetrasaccharides and changes in CD with pH revealed striking difference in apparent pks; and c) binding of active heparin chain to antithrombin involved a run of specific negative charges beyond the active tetrasaccharide and promoted local conformational change involving tryptophan, possibly tyrosine residues, and a small change in overall protein conformation.

Another visible role for basic studies of molecular biology of heparin was seen in the activation of tyrosine hydroxylase (TH), the enzyme responsible for DOPA formation by heparin. Furthermore, the enzyme is believed to exist in forms of "low" and "high" enzymatic capacity. Our studies defined a rational approach to selecting agents which might affect this enzyme system in a predicted manner. I have studied the regulation of striatal tyrosine hydroxylase by heparin-like glycosaminoglycans reexploring the molecular characteristics and specificity of the interaction using a model for the molecular basis of the activation analogous with that of the antithrombin system. This was characterized in three important ways; 1) specificity of heparin; 2) interaction of heparin with lysyl groups on the protein and 3) a high potency fraction of about 10 percent of the bulk and an increase in specific activity of high molecular weight active chains. Partially purified mouse striatal tyrosine hydroxylase, (up to 10 fold purified in a one-step heparin-affinity column) was used as the source of the enzyme; heparin, heparin fractions, heparan sulfate and other polyanions were looked at in regard to 1) and 3) in the direct activation of TH, while the putative reaction with lysyl residues was investigated indirectly using P(L:T) and P(L:P) as competitive protein surfaces. Three findings of importance emerged, 1) one of our heparin fractions, (high molecular weight, separated by mass, and 5% of the total) was significantly more potent on an equivalent charge basis than the bulk preparation and major fraction; 2) heparin sulfate did not activate tyrosine hydroxylase at the same concentration of charge despite its heparin-like components, nor did sulfated keratan sulfate, another glycosaminoglycan of the heparin class (1969); and 3) although P(L:T) and P(L:P) inhibited the activation of TH by heparin they proved to be stronger inhibitors of the enzyme directly, in the absence of heparin, which reversed the inhibition; a potent cationic peptide inhibitor of the enzyme was developed, i.e., lysyl-tyrosine amide, and employed in a one-step affinity-purification (5-7 fold) of enzyme (A-TH) which had an 8-fold lower Ki for inhibitor than that of the heparin affinity preparation (H-TH).

The analogy with the antithrombin system was, therefore, partially supported by the results (1) and 2), which was consistent with activation by decreasing the Km for cofactor) but heparin appeared better to relieve inhibition (desensitize the inhibited enzyme to the fully activated state) than to cause the direct activation 3) (which was consistent with the failure to further decrease the low Km for tyrosine). H-TH has been repeatedly shown not to exhibit substrate (tyrosine) inhibition with tetrahydrobiopterin as cofactor. However, L:T markedly raised the apparent Km for tyrosine from about 15 to 125 μMolar in kinetic studies indicating mixed-type of competitive inhibition. Heparin, in desensitizing the enzyme from the inhibition, caused a decrease in the apparent Km for tyrosine towards that of the activated form. The data showed that TH could be regulated by two mechanisms as revealed by heparin activation, 1) occupancy or vacancy of a positive region by an anionic effector would control the Km for cofactor, while 2) occupancy or vacancy of a negative site by a cationic inhibitor would determine the apparent Km for tyrosine. These results bear on possible regulatory properties of tyrosine hydroxylase in vivo, especially in regard to transitions between forms of low and high enzymic capacity.

The proposed course of the project is a continuation of the basic studies of glycosaminoglycan-polypeptide interactions. Studies on the regulation of striatal tyrosine hydroxylase by heparin-like glycosaminoglycans of the CNS will include comparison of TH activation by various AT-active and inactive heparin fractions and segments, and by high concentration of selected fractions of heparin sulfate; new procedures for purification of enzyme will continue to be developed using our knowledge of the heparin-TH interaction.

Collaboration with Dr. Robert Rosenberg will continue as we attempt to identify the unique ring structure of the iduronic acids in the active site and the specific oligosaccharide conformation which leads to the abnormal pK. Measurement of the impact of these heparin segments (and possible model compounds) on the conformation of AT and polypeptide models will complement these structure-function studies. We are also trying to develop a chemical method for splitting the heparin molecule in a predictable manner which will yield good quantities of the active site region - to replace the laborious enzymatic-low yield procedures currently employed.

The methods are measurements of molecular asymmetries by extrinsic and intrinsic Cotton effects using the Cary Models 6001-63 circular dichroism apparatus. The metachromatic reactions developed earlier in the project (measured spectrophotometrically and by circular dichroism) provide a quantitative measure of the degree of amine interactions with anionic sites and their geometry. Computer models for determination of the proportion of α-helical, β- or non-ordered structure in the polypeptide reveal the degree and kind of conformation induced by a given PS. Heparin fractions were obtained by mass separation on Sephadex G-200 chromatography columns and by affinity chromatography with antithrombin.

Studies of striatal tyrosine hydroxylase utilize dissection of mouse striatum and partial purification by means of a one-step separation by heparin-affinity chromatography. Standard radioactive assays are used to determine TH activity.

Heparin activity is measured in vitro using highly purified protein components (AT, thrombin, etc.) and a fluorometric assay based on the increase in tryptophane fluorescence of AT upon heparin binding.

Metachromatic measurement of heparin charge 1) determined the degree of protonization of the uronic acids of heparin as a function of decreasing pH and 2) showed that the stoichiometry was 1 to 1 with positive lysyl residues for poly(L-lysine:L-tyrosine) and close to 1 to 1 with poly(L-lysine:L-phenylalanine). The stoichiometry and interactions of heparin fractions with poly L-lysine:tryptophan can be sensitive measurements of structure-function relationships of this potent anticoagulant biopolymer.

Honors:

Elected to the Advisory Board of the journal Biopolymers commencing January, 1980.

Guest speaker for the session on "Heparin - Physical and Chemical Properties" in the Symposium on Chemistry and Biology of Heparin, March 21-22, 1980, sponsored by the American Heart Association Councils on Thrombosis and Arteriosclerosis, presenting the paper entitled "Physical-Chemical Characterization of Heparin Fractions".

Guest speaker for the session on "Heparin - Molecular Biology" in the symposium (see above), to present the paper entitled "Molecular Basis for the Activation of Tyrosine Hydroxylase by Heparin".

Invited to join the Council on Thrombosis and Hemostasis (Council on Basic Sciences) of the American Heart Association, April, 1980.

Publications:

Stone, A.L.: Studies on a molecular basis for the heparin-induced regulation of enzymatic activity of mouse striatal tyrosine hydroxylase in vitro. Inhibition of heparin activation and of the enzyme by poly-L-lysyltyrosine and poly-L-lysylphenylalanine and their constituent peptides. J. Neurochem., in press.

Stone, A.L.: Physical-Chemical Characterization of Heparin Fractions. In Lundblad, R.L. and Mann, K.G. (Eds.): Chemistry and Biology of Heparin. New York, Elsevier North Holland, Inc., 1980, in press.

Stone, A.L.: Molecular Basis for the Activation of Tyrosine Hydroxylase by Heparin. In Lundblad, R.L. and Mann, K.G. (Eds.): Chemistry and Biology of Heparin. New York, Elsevier North Holland, Inc., 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01037-12 LNC

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Role of the Cell Membrane in Cellular Organization, A Molecular Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Neville, Jr.	Chief, Sect. on Biophys. Chem.	LNC NIMH
OTHER:	Wyrta Heagy	Staff Fellow	LNC NIMH
	Richard Youle	Staff Fellow	LNC NIMH
	Gary Murray	Visiting Associate	LNC NIMH

COOPERATING UNITS (if any)

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Laboratory of Neurochemistry

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Biophysical Chemistry

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein hybrids containing the active fragment of a toxin and another receptor specific binding protein. Such artificial protein hybrids may have value as a new class of pharmacologic reagents.

PHS-6040
(Rev. 10-76)

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to synthesize a new class of pharmacologic reagents which permit the modulation of specific cell types in a manner unobtainable with conventional reagents. This is being accomplished by the synthesis of artificial protein hybrids which utilize the process of receptor-mediated protein transport.

A wide variety of proteins are capable of entering cells by receptor-mediated transport processes. Having gained entry these proteins are directed to specific cellular compartments where they exert either a physiological or pathological function.

In general it appears that only a discrete portion of these proteins contain the receptor binding activity which is involved in the entry process while another portion of the protein performs the intracellular function. Therefore, it is possible to split and reassemble two such proteins with a new combination of receptor entry specificity and intracellular function. Such proteins we call artificial hybrid proteins, and previous reports from this laboratory have suggested that such hybrids should have utility both as probes of entry processes and as a new class of pharmacologic reagents with tailor made receptor and therefore cell type specificity.

Clinical Applications:

A hypothetical example of the clinical utility of an artificial hybrid protein reagent is the following. Many malignant tumors are known to carry tumor specific receptors on their cell surfaces (tumor specific antigens). An artificial hybrid protein containing a functional group binding to these specific receptors and linked to a highly toxic intracellular functional group (diphtheria toxin fragment A) might function as a tumor specific toxic reagent. The advantage of this type of reagent over currently available antitumor drugs would be the property of cell type specificity.

Artificial hybrid protein technology also holds the promise for constructing new types of neuropharmacologic reagents. Tetanus and botulinum toxins inhibit the release of inhibitory and excitatory neurotransmitters respectively, by virtue of their intracellular functional A chains. By linking these A chains to proteins which bind to only certain areas of the brain, a high degree of regional specificity might be achieved, and it is likely that the regional specificity would be mirrored by behavioral specificity.

Major Findings:

1. Previously we reported the construction of a cell type specific toxic agent by modifying the receptor specificity of the toxin ricin by covalent binding mannose-6-phosphate and reversibly binding lactose. This

work has now been extended using a monoclonal antibody directed against the murine T cell adult differentiation antigen Thy 1.2.

Anti-Thy 1.2-ricin in the presence of lactose specifically kills normal cells and tumor cells carrying Thy 1.2 antigen. Cells not carrying Thy 1.2 are only affected at 700 fold higher concentrations of anti-Thy 1.2-ricin. This degree of selectivity is sufficient to utilize anti-Thy 1.2-ricin as an anti-tumor agent in vivo to treat experimental murine leukemia and lymphoma.

2. Proteins which fail to enter cells due to loss of receptor-mediated protein transport systems secondary to disease states, can be rerouted into the cell via alternate receptors. Thus, mannose-6-phosphate-low density lipoportein is taken up by cells cloned from patients with familial hypercholesterolemia which lack the low density lipoprotein receptor. Low density lipoprotein entering these cells by the new route functions in a physiologic manner and down regulates the rate limiting enzyme in cholesterol synthesis.

These findings show that it is possible to construct cell type specific reagents by utilizing hybrid protein technology. The overall goals of the project therefore appear to be sound.

Publications:

Youle, R.J. and Neville, D.M., Jr.: Receptor-mediated transport of the hybrid protein ricin-diphtheria toxin fragment A with subsequent ADP-ribosylation of intracellular elongation factor II. J. Biol. Chem 254: 11089-11096, 1979.

Youle, R.J., Murray, G.J. and Neville, D.M., Jr.: Ricin linked to phosphomannan binds to fibroblast lysosomal hydrolase receptors resulting in a cell type specific toxin. Proc. Natl. Acad. Sci. USA 76: 5559-5562, 1979.

Youle, R.J. and Neville, D.M., Jr.: Anti-Thy 1.2 monoclonal antibody linked to ricin is a potent cell type specific toxin. Proc. Natl. Acad. Sci. USA in press.

Murray, G.J. and Neville, D.M., Jr.: Mannose-6-phosphate receptor mediated uptake of modified low density lipoprotein results in down regulation of HM6 CoA reductase in normal and familial hypercholesterolemic fibroblasts. J. Biol. Chem. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01038-09 LNC

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Development of Mutant Mice for Phenylketonuria and Dihydropteridine Reductase Insufficiency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Audrey L. Stone	Research Chemist	LNC NIMH

COOPERATING UNITS (if any)

Section on Myelin Chemistry, Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

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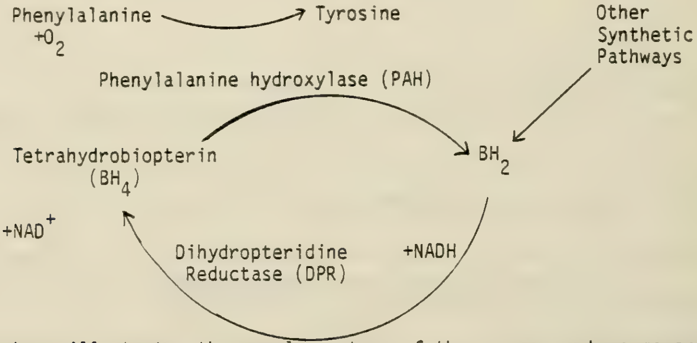
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The project is a small program to develop a murine model for PKU and/or related diseases. Another objective is the elucidation of regulatory mechanisms in the expression of phenylalanine hydroxylation (PH). Criteria for a putative deficiency in PH had been established: plasma phenylalanine levels, plasma phenylalanine-to-tyrosine (P/T) ratio, and hepatic levels of dihydropteridine reductase (DPR). Early results indicated that hepatic DPR in mice was not in great excess, contrary to generally accepted hypotheses. Murine DPR insufficiency was expressed by mild symptoms of deficiency in PH and was studied using mildly elevated P/T as a criterion. Putative mutant families were developed, but infertility and/or selective survival led to a loss of viable abnormal offspring at F_4 or higher. Current work reports the relationship of in vivo elevated P/T to in vitro measurable hepatic phenylalanine hydroxylase (PAH) using parachlorophenylalanine to inhibit PAH in vivo, and examines pharmacologically-induced murine models for dihydropteridine reductase deficiency.

PHS-6040
(Rev. 10-76)

Project Description:

The lack of appropriate animal models for the study of enzyme-deficient genetic diseases associated with mental retardation has checked the search for the pathophysiology directly relating the CNS lesion with the enzyme defect. This project was started as a single-laboratory size program to obtain a murine model for the human disorders related to phenylketonuria (PKU). Suspect heterozygote mutant offspring were generated using double-dose X-irradiation of mouse sires (see previous reports, M-NC-8, 1972-74). The offspring were screened for the defect in a manner similar to the human screening program, using blood plasma levels of phenylalanine (P), tyrosine (T), and their ratio (P/T) to distinguish abnormal, high values in possible mutants from a distribution of values obtained from mice with normal phenylalanine hydroxylation (PH) systems here shown:



The scheme illustrates the complex nature of the enzyme systems governing the steady-state levels of the amino acids in the blood (two enzymes, a cofactor and a coenzyme). Thus, the screening program, in principle, would detect a deficiency in any component shown or a change in amino acid levels due to other causes. At the onset of the project the dogma indicated that dihydropteridine reductase (DPR) was in such excess as to permit normal levels of hydroxylation in the heterozygote mutant deficient in this component. The program included, therefore, screening of a large sample of liver biopsies for direct measurements of hepatic DPR. Total dihydrobiopterin in hepatic samples was measured.

Over the course of several years the project has been limited by short supply of personnel to carry out its aims vigorously. The thrusts of the results have been 1) achievement of a pharmacological model for the suspect "PKU" mouse with criteria for screening; 2) selection of six offspring suspects for deficiency in phenylalanine hydroxylation 4 of which were shown to have DPR insufficiency and were carried to F_4 generation where infertility led to loss of viability of the strains and 3) limited in vitro kinetic studies of the new DPR inhibitor (see Z01 MH 01038-07

MH, 1977-78) with the promise of a pharmacologically-induced murine DPR deficiency. This project applied various modes of administration of parachlorophenylalanine (pCPA) to mice, and measured the time course of uptake of the agent into blood plasma and liver, with corresponding in vitro enzyme assays for hepatic PAH. A program for separation of phenylalanine, tyrosine and pCPA from blood plasma and liver extracts at various times after administration of reagent was developed for the automated amino acid analyzer in collaboration with Gladys Deibler, LCM, NIMH. Protein contents of liver samples were determined by colorimetric analysis.

Eighteen hours after the intraperitoneal administration of 360 mg/kg pCPA in mice scant amount was detected in the blood or liver. The maximum level was reached at 3-6 hours and falls. Doubling the dose led to approximately twice the maximal concentration in liver and blood from 3-6 hours and falling close to zero by 18 hours. The animals excreted large quantities of phenyl ketones during the 12 hour period. In vitro measurable PAH levels fell during the 6-18 hour period, using the natural cofactor (BH_4) or the synthetic ($DMPH_4$), confirming our previous findings at 24 hours with $DMPH_4$ (see previous report N-MC-8 1974). The inhibition curve was bimodal between zero and 24 hours. The maximal decrease in hepatic phenylalanine hydroxylase activity compared with normal during 0-36 hours was found to 45-50%. Plasma tyrosine level decreased, phenylalanine increased and the P/T ratio increased 1.5-3 fold during the zero to 9 hour period.

A new mode of administration of pCPA was tried with the view towards achieving high levels of pCPA in the blood and liver over longer periods. This was successful. Maximal plasma levels were about 4 fold higher than previously and persisted over 24 hours.

The remaining, aged nonfertile 4th generation of suspect DPR-mutant families exhibited diminished in vitro measurable PAH activity at sacrifice (25-80% of controls) (see previous reports MNC 8, 1972-76). It was further shown that in vitro measurable PAH activity was similar in young adults and aged exbreeders from the normal NIH colony. The data further confirm the original premise that small increases in P/T in mice is a reflection of diminished PH activity in vivo due to either DPR insufficiency or decrease in phenylalanine hydroxylase. The suspect mutants, therefore, have been established to exhibit enzymatic and biochemical characteristics of the human genetic disorder of Dihydropteridine Reductase Deficiency. We explain the failure to maintain the substrain by the normal reproductive methods as due to impairment of the hormonal regulations of mating, nesting, fertility and/or lactation/care behavior in mice since both male and female affected animals often survived with the deficiency beyond one year of age. In one case of 8, hormone treatment induced fertile mating and viable offspring.

Kinetic studies with purified enzyme in vitro showed that the DPR inhibitor was an order of magnitude more potent than methotrexate using the natural cofactor BH_4. This indicates its eminent suitability for in vivo administration and inhibition of DPR to produce DPR deficiency in mice, with the caviat that folate intermediates be administered at the

Methotrexate inhibits phenylalanine hydroxylation in mice via its role as an inhibitor of DPR. The inhibition was limited to up to 50 percent at relatively high doses.

The results are significant to an understanding of the effects of pCPA in mice and modes of inhibiting PH *in vivo* in mice. Parameters of PH insufficiency in mice such as P/T ratio are now quantitatively related to the degree of impairment of hepatic PAH. Such knowledge is important to the development of a murine strain which can serve as an animal model for various inborn diseases affecting the nervous system.

The studies of murine DPR deficiency and the pharmacologically-induced state of DPR insufficiency (and preparation of appropriate manuscripts) will be continued, - space, equipment and personnel permitting.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01081-10 LNP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Role of Motor Cortex and Red Nucleus in Control of Voluntary Movement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.45	.65	1.80

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves utilization of single neuron recording and operant conditioning techniques in behaving monkeys to study brain mechanisms underlying voluntary movement. Monkeys are trained to make precise movements of a handle whose rotation controls a visual display, and stimuli are delivered via the handle by means of an electronically controlled torque motor"in order to determine how sensory feedback is processed. Red nucleus neurons (RNNs) failed to show changes in frequency preceding EMG changes of arm muscles discharging in advance of large, high velocity movements, with RNN discharge starting at movement onset or during movement and peaking near movement termination. In contrast to this terminal peaking of RNN discharge, the activity of the muscles usually peaked around movement onset, and early peaking was also seen for both precentral and postcentral pyramidal tract neurons. The observed RNN discharge patterns fit nicely with their putative role in regulation of reflex excitability with different phases of movement.

PHS-6040
(Rev. 10-76)

Project Description:

In the previous work of this project on motor cortex, primary attention has been focused on a class of motor cortex neurons which send their axons to the spinal cord via the pyramidal tract. These neurons are referred to as pyramidal tract neurons (PTNs) and are of particular importance because they convey the final output from motor cortex to the spinal cord regions from which impulses pass out to the muscles to generate movement. In recent experiments, a second important class of motor cortex output elements has been investigated. These are the motor cortex neurons which send their axons from the cortex to the red nucleus, which is a collection of cells which forms a link between cerebral cortex and spinal cord. Unlike PTNs, the cortico-red nuclear neurons (CRNs) pass only as far as the brain stem, and it is the cells lying in the red nucleus whose axons then pass to the spinal cord. Within the red nucleus, impulses from the cerebral cortex are integrated with impulses reaching the red nucleus from the cerebellum.

In the work which has been done in the past year, we first identified zones within the red nucleus receiving projections from the motor cortex arm area in the monkey. Having identified this area and determined the properties of cells, we then permanently implanted stimulating electrodes within the arm area of the red nucleus, and used these stimulating electrodes to excite the axon terminals of fibers reaching the red nucleus from the arm area of the cerebral motor cortex. In these experiments, microelectrodes were used to record impulses of single nerve cells in the arm area of the motor cortex while electrical stimuli were delivered within the red nucleus to identify that class of motor cortex neurons which sends its axons to the red nucleus. Having identified these CRNs, we then studied their discharge properties during movement and compared them to the discharge properties of PTNs.

Major Findings:

I. Red Nucleus

Recordings of the activity of red nucleus neurons (RNNs) revealed that they exhibit selective involvement in processes controlling movement termination. In this study, RNN discharge was recorded in a monkey rewarded for maintaining a handle in one of three positions (15° pronation, neutral, 15° supination) on the basis of a visual display. A shift of the visual display triggered active 15° pronation or supination movements between these three positions. A torque motor coupled to the handle allowed variation of steady state load in each position and provided a means of delivering supinating or pronating ramp displacements to test afferent responsiveness of RNNs.

One-hundred sixty of 229 RNNs recorded from the magnocellular and posterior parvocellular RN showed relations with some aspect of the task, and 34 of these 160 were highly related, i.e., their frequency changed even with the very small corrective supinations or pronations (1.5° or less in amplitude) performed in this paradigm during steady holding in a given position. For most RNNs, changes in frequency failed to precede EMG changes of arm muscles discharging in advance of large, high velocity movements, with RNN discharge starting at movement onset or during movement. The typical time course of RNN discharge (observed for 24 of the 34 highly related units) during the 15° movements was a gradual build-up of activity with peaking near termination. In contrast to this terminal peaking of RNN discharge, the activity of the muscles usually peaked around movement onset, and early peaking was also seen for both precentral and postcentral PTNs. Unlike the majority of sensorimotor cortex PTNs, RNN discharge did not vary with different levels of muscle activity in the steady state due to different steady state loads or different steady state holding positions. Another difference between RNNs and PTNs was the relatively low incidence of short-latency afferent responses to passive ramp displacements in RNNs, especially RNNs related to small active movements. Only 8 of the 34 units related to small movements had response latencies less than 50 msec, whereas 20/34 of these units had long-latency responses (>100 ms) to the passive ramp displacements.

Many RNNs failing to discharge prior to large, high velocity movements nevertheless discharged prior to small corrective movements where the starting point of the movement was already close to the terminal point. In view of the findings of previous investigators concerning the action of RN on dynamic gamma motoneurons, it seems possible that accurate termination of a movement involves an increased bias upon the dynamic gamma motoneurons, while the initial acceleration is accompanied by a relative decrease of the dependence on the velocity parameter in the agonist myotatic loop. RNN discharge patterns fit nicely with this putative role in differential regulation of reflex excitability with different phases of movement.

II. Cerebral Motor Cortex: Differences between Motor Cortex Pyramidal Tract Neurons (PTNs) and Corticorubral Neurons (CRNs).

Following microelectrode recordings from red nucleus (RN), stimulating electrodes were permanently implanted in RN (two pairs of electrodes) and in PT (one pair) to allow antidromic identification of three classes of motor cortex neurons: PTNs, CRNs and PT-CRNs (i.e., neurons with branches to both PT and RN). CRNs commonly had conduction velocities ranging from 8-20 m/s and were usually antidromically driven by the RN electrode pair which was situated at the dorsolateral boundary of the posterior parvocellular RN. The PT-CRNs belonged mostly to the fast subset of the PTN population (50-60 m/s) and were more often driven by stimulation of the RN electrode pair situated at the caudal pole of the magnocellular RN. Twenty-two CRNs, 27 PT-CRNs and 196 PTNs were extensively studied using the same paradigm as in the prior RN recordings. In general, the low frequency irregular discharge and the rather slight changes of discharge with active movement shown by the CRNs were markedly different from the regular tonic discharge and intense movement-related modulation of the PTNs. In order to compare CRNs and PT-CRNs with PTNs in exactly the same motor cortex loci, analyses were restricted to a subgroup of 98 PTNs which had been recorded in the same 38 penetrations with the CRNs and PT-CRNs. A major difference between the three subsets was in their time of discharge with respect to movement onset: 90% of the PTNs changed frequency before start of the movement and 10% at or after movement, whereas only 26% of CRNs changed before movement, the remaining 74% showing changes only at or after movement onset; the PT-CRN population was intermediate. This same conclusion was reached by comparing timing of neurons within each of the individual penetrations (Ps) taken separately. For 10 Ps, PTNs clearly preceded CRNs, with only 3 Ps showing overlap and no Ps showing CRNs prior to PTNs. For 9 Ps, PTNs preceded PT-CRNs and in 8 Ps, PTNs and PT-CRNs overlapped. The three subgroups also differed with respect to effects of different steady state loads: whereas different loads were associated with marked changes of discharge frequency in a majority (67%) of PTNs, the effects of different loads were slight and found in only 30% of PT-CRNs and virtually absent in CRNs. Another difference was that the incidence of short (<50 ms) latency responses to passive ramp displacements was lower in the CRNs (23%) as compared to the PTNs (62%).

These findings point to clearly different functional roles for CRN and PTN outputs from motor cortex, and are consistent with the characteristics of RN discharge observed during prior RN recordings in the same monkey.

Interpretations and Conclusions:

In thinking of central motor programs, it is useful to imagine the case of a person reaching out to touch something located an arm's length away: there is an initial large, relatively high velocity movement and then, when the hand is near its goal, small, relatively low velocity adjustments are made as the movement terminates. The initial, high velocity phase of the movement involves open-loop control, while the terminal adjustments use feedback. Open-loop control is used when the difference between the starting position and the terminal position is large and, if well-calibrated, the open-loop controller will quickly move the limb close enough to the terminal position so that the error becomes small enough for feedback to function effectively. The notion of open-loop control implies a strategy which will rapidly bridge large discrepancies from desired position at too great a velocity for feedback paths to function effectively, and rather than lumping feedforward and feedback into a single controller, it has been suggested that closed-loop and open-loop controls involve separate control strategies and thus may have separate structural embodiments.

It has been proposed that for heuristic purposes it may be useful to identify two main groups of functions for movement initiation and control: (1) the first group would deal with changes of setpoint; (2) the second group would deal with "...adaptive features of the loop at a given operating point, taking into account peripheral conditions." It seems that the first group of functions depends primarily upon the sensorimotor cortex, while the second group of control functions depends in large part upon the intermediate cerebellum and related structures. One structure which is linked to the intermediate cerebellum (via the nucleus interpositus) is the red nucleus, from which we have recorded neural activity in the past year.

These ideas concerning red nucleus and closed-loop control provide a background for our observations that red nucleus neurons (RNNs) are selectively involved in control of movement termination. We have also found that there are striking differences in the onset times of motor cortex PTN discharge and RNN discharge prior to large, high velocity movements: PTN discharge begins well in advance of the burst of muscle activity occurring with high velocity movements and peaks prior to peak muscle activity whereas RNN discharge builds up gradually during movement and peaks near movement termination. Furthermore, PTN responsiveness to limb displacement varies depending on whether the displacement occurs in the early, high velocity phase of movement (when responses are attenuated) or during precisely controlled small movements (when responses are enhanced). These changes in PTN responses depending on movement strategy are consistent with the notion that the high velocity phase is relatively open-loop, while the fine adjustments are more influenced by feedback.

In addition, we have studied activity of two other categories of antidromically identified motor cortex neurons in the same monkey and same paradigm: CRNs (cortico-rubral neurons) and PT-CRNs (neurons whose axons project both to red nucleus and to pyramidal tract). It was found that the properties of CRNs correspond to those of RNNs, while the properties of PT-CRNs are intermediate between those of CRNs and PTNs.

Proposed Course:

Within the next year we intend to extend these studies on RNNs and PTNs, since we have thus far investigated an insufficient number of animals to allow us to draw final conclusions.

Significance to Biomedical Research and to the Program of the Institute:

This project seeks to apply basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in red nucleus, basal ganglia, and cerebellum.

Publications:

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Evarts, E.V.: Brain control of movement: possible mechanisms of functional reorganization. In Bach-y-Rita, P. (Ed.): Recovery of Function: Theoretical Considerations for Brain Injury Rehabilitation. Baltimore, University Park Press, 1980, pp. 173-186.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01084-09 LNP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Eye-Head Coordination in the Primate: Neural Substrate and Plasticity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	2.0	1.40

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range aim of this project is to determine the neural basis of adaptive mechanisms responsible for maintaining appropriate performance levels in the oculomotor system. Mechanisms currently under study relate to 1) the gain of the vestibulo-ocular reflex, 2) the pulse-step ratio of saccadic eye movements and 3) the gain of accommodation-vergence. One series of experiments showed that bilateral flocculectomy in the monkey results in severe deficits in the first two of these adaptive mechanisms and also in the optokinetic response. A second series demonstrated that changes in the gain of the vestibulo-ocular reflex result in parallel changes in the optokinetic response. A third series showed that the compensatory eye movements generated by the vestibulo-ocular reflex following sudden disturbances of the head are essentially uninfluenced by ongoing tracking behavior in the short-term; however, prolonged foveal pursuit during head movements--in the absence of any peripheral retinal slip--can gradually elicit long-term changes in the gain of the vestibulo-ocular reflex.

Objectives:

Our continuing objective is an understanding of the adaptive mechanisms responsible for maintaining appropriate performance levels in the oculomotor system. Three mechanisms are presently under study: (1) the regulation of the gain of the vestibulo-ocular reflex; (2) the maintenance of an appropriate match between the static and dynamic components of saccadic eye movements; and (3) a newly-discovered adaptive process that is responsible for regulating the gain of accommodation-vergence. One series of experiments on monkeys was undertaken to determine the effects of bilateral ablation of the floccular lobes of the cerebellum on these adaptive capabilities and also on the optokinetic response. A second series concerned the effects of changes in the gain of the vestibulo-ocular reflex on other systems generating smooth eye movements (pursuit and optokinetic). A third series concerned the magnitude of the compensatory eye movements associated with sudden disturbances of the head during combined eye-head tracking. Lastly, experiments were performed to determine whether gross retinal slip was necessary to induce large adaptive changes in the gain of the vestibulo-ocular reflex.

Methods:

The adaptive control of the gain of the vestibulo-ocular reflex and of the pulse-step ratio of saccades was studied using previously described techniques. Briefly, the gain of the vestibulo-ocular reflex was increased by prolonged exposure to x2 telescopic spectacles or decreased by exposure to goggles providing a visual field that was fixed with respect to the head. The pulse-step ratio of saccades was altered by means of optically-imposed post-saccadic retinal slip.

A new optical technique was devised to determine whether the system responsible for accommodation-vergence possesses an adaptive capability. The operation of the accommodation-vergence system is most readily appreciated in a monocular viewing situation: as the viewing eye focuses on targets at closer and closer distances (accommodates), the covered eye converges and so tends to keep aligned with the object of regard. Thus, accommodation in one eye elicits vergence in the other, and the "gain" of this response can be expressed as the actual ocular convergence divided by the ocular convergence that would be required to maintain ideal binocular alignment. The Maddox rod technique was used to determine the alignment of the non-viewing eye, and the "gain" of accommodation-vergence was determined using targets at 12 different viewing distances. Subjects wore laterally-directed periscopic spectacles to increase the separation of the two lines of sight and thereby increase the apparent separation of the eyes. This optical arrangement increases the magnitude of the vergence eye movements that must accompany a given change in accommodation in order to maintain binocular alignment on the object of regard (single, fused image). If the system has an adaptive capability, it should respond to such an optical challenge by increasing its "gain". The latter studies were done in collaboration with Dr. Stuart Judge of the Laboratory of Sensorimotor Research in the National Eye Institute.

Bilateral flocculectomies were performed by Dr. David S. Zee at Johns Hopkins University on two monkeys using suction ablation under direct visualization.

Horizontal optokinetic responses were elicited by seating monkeys inside a full-field striped cylinder that rotated at constant velocity; the resulting nystagmus consists of smooth tracking eye movements in the direction of stripe movement, interrupted at intervals by resetting saccades. After sudden illumination of the moving stripes, the velocity of the tracking eye movements shows an initial rapid rise (lasting a few hundred milliseconds) followed by a more gradual increase (lasting 10-15 sec); these rapid and gradual events are believed to represent two separate components of the optokinetic response. After the illumination goes out, there is an initial rapid decrease in smooth eye velocity followed by a long-lasting afterresponse (so-called optokinetic afternystagmus), the initial velocity of which is assumed to be an index of the level achieved by the gradual component of the prior optokinetic response.

Attempts were also made to induce changes in the gain of the vestibulo-ocular reflex in the absence of peripheral retinal slip. Eye movements were recorded in a monkey that was rewarded for fixating a small light in totally dark surroundings. During fixation, the light and the monkey were oscillated in the horizontal plane either in-phase or 180° out-of-phase, simulating the eye-head movement relationships normally associated with low- or high-gain spectacles.

Major Findings:

1. Thirty minutes of exposure to laterally-directed periscopic spectacles was sufficient to induce large increases (up to two-fold) in the gain of the accommodation-vergence response in both human subjects and rhesus monkeys. This strongly suggests that accommodation-vergence is normally subject to adaptive regulation.

2. Bilateral flocculectomy has profound effects on the monkey's visual following mechanisms and also on the adaptive capability of its oculomotor system:

(i) After flocculectomy, the rapid component of the optokinetic response was considerably attenuated (open-loop gain, measured as eye acceleration divided by retinal slip, was only 25% of normal), while the gradual component achieved nearly normal amplitude but over a much longer time course. Thus, saturation of the gradual component (as indicated by the maximum initial velocity of the optokinetic afternystagmus) was almost normal, while the "charging" time constant of the afternystagmus was 2-4 times normal.

(ii) Before flocculectomy, one day of passive oscillation at 0.2 Hz with x0 spectacles caused the gain of the vestibulo-ocular reflex to drop from 1.00 to 0.49 in one animal and 0.98 to 0.55 in the other. After flocculectomy, the vestibulo-ocular reflex of both monkeys had low gain (0.92 and 0.76, which persisted for four months) and showed reduced adaptability: e.g., even after two days of passive oscillation at 0.2 Hz with x0 spectacles, the gains fell to only 0.78 and 0.64, respectively.

(iii) The animal's ability to adapt to optically-imposed post-saccadic retinal slip was estimated from changes in the saccadic pulse-step mismatch, defined as amplitude of post-saccadic ocular drift divided by the magnitude of the antecedent saccade. Exponential slip imposed in the direction of the antecedent saccade (with a 50-msec time constant and 50% of the saccade's amplitude) resulted in large, adaptive changes in the pulse-step mismatch before the lesion (from -3% to -70% in one animal and -1% to -50% in the other), while afterwards, these changes were considerably reduced (from -2% to -7% and +1% to -5%, respectively).

(iv) Accommodation-vergence and its adaptive modification by periscopic spectacles were not significantly different from normal following flocculectomy.

3. Six-fold changes in the gain of the vestibulo-ocular reflex (0.29 to 1.80) were associated with parallel, 4.5-fold changes in the initial eye velocity of optokinetic afternystagmus, but little or no change in two measures of the rapid component of the optokinetic response (eye acceleration in the first 100 msec and peak eye velocity achieved in the first second). That the rapid and gradual events of the optokinetic response are differentially affected by changes in the gain of the vestibulo-ocular reflex supports the idea that they represent separate components mediated by separate neural pathways. Furthermore, these findings imply that the signals responsible for the gradual component achieve access to the final oculomotor pathways through the modifiable elements subserving changes in vestibulo-ocular reflex gain while those mediating the rapid changes in eye velocity enter elsewhere downstream.

4. Sudden, unexpected disturbances of the monkey's head elicited compensatory eye movements whose magnitude was independent of the ongoing pursuit behavior during combined eye-head tracking. In particular, the compensatory eye movements were not "suppressed" in situations involving pursuit of targets moving with the head. Changes in the gain of the vestibulo-ocular reflex following prolonged exposure to telescopic spectacles or fixed-field goggles resulted in proportionate changes in the magnitude of the compensatory eye movements associated with these sudden disturbances; again, these were uninfluenced by any ongoing tracking behavior. These observations are consistent with the view that in the short-term, the gain of the vestibulo-ocular reflex is fixed and immutable and, under all circumstances, the reflex functions to preserve the pre-existing gaze velocity.

5. Eight hours of coordinated foveal pursuit during head movement against dark surroundings caused adaptive changes in the gain of the vestibulo-ocular reflex as large as 50% of those seen after equivalent passive oscillation in spectacles. That foveal pursuit alone during head movement is sufficient to elicit changes in the vestibulo-ocular reflex gain is consistent with the view that central correlates of pursuit might furnish part of the error signal guiding the adaptive process. This might explain why lesions of the

flocculus--an important element of the pursuit system--compromize adaptive gain control in the vestibulo-ocular reflex even though our single unit evidence suggests that the flocculus is not the site of the modifiable elements.

Significance to Biomedical Research and the Program of the Institute:

A great deal is known about the anatomy and physiology of eye movements. The discrete, rather machine-like quality of the oculomotor system has proved particularly amenable to the analyses of information processing at the level of the system and the single neuron. The fact that the system also displays plasticity opens up new approaches to the subject of motor learning at the cellular level in the primate central nervous system. That the alignment of the two eyes, under the influence of accommodation-vergence, is subject to adaptive regulation is especially interesting since by far the most common clinical disorders of eye movements involve misalignment of the two eyes with respect to one another. The new optical techniques used in the present study were based on a systems theory approach to the regulation of eye movements rather than the "exercise approach" usual in conventional orthoptics; they may lead to new optical techniques that are of therapeutic value in the treatment of binocular misalignments in human patients. The oculomotor deficits that follow lesions of the flocculus in the monkey demonstrate the necessity for the functional integrity of this region of the cerebellum for normal visual following and adaptive regulation in the vestibular and saccadic systems. These findings also suggest further tests that might aid localization of the pathology in human patients with classical cerebellar signs.

Proposed Course:

The adaptive capability of the oculomotor system will continue to be the main concern in these studies. Single unit studies will be employed in the search for the locus of the modifiable elements in the vestibulo-ocular reflex and will concentrate on the later stages of the disynaptic vestibular pathway. Electrophysiological techniques will also be employed in the floccular lobe of the cerebellum to determine any correlation with the adaptive mechanisms responsible for regulating the pulse-step ratio of saccadic eye movements. The newly-discovered adaptive mechanisms in the accommodation-vergence system will be examined more closely to determine time constants of acquisition and decay; studies will also be initiated in human strabismic patients to determine whether they possess such adaptive capabilities.

Publicatons:

Miles, F.A.: Information processing at the cellular and systems level in complex organisms. In: Pinsker, H.M., and Willis, W.D. (Eds.): Information Processing in the Nervous System. New York, Raven, 1980, pp. 313-329.

Miles, F.A., and Eighmy, B.B.: Long-term adaptive changes in primate vestibulo-ocular reflex. I. Behavioral observations. J. Neurophysiol. 43: 1406-1425, 1980.

Miles, F. A., and Braitman, D.J.: Long-term adaptive changes in primate vestibulo-ocular reflex. II. Electrophysiological observations on semicircular canal primary afferents. J. Neurophysiol. 43: 1426-1436, 1980.

Miles, F.A., Fuller, J.H., Braitman, D.J., and Dow, B.M.: Long-term adaptive changes in primate vestibulo-ocular reflex. III. Electrophysiological observations in flocculus of normal monkey. J. Neurophysiol. 43: 1437-1476, 1980.

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Lisberger, S.G., and Miles, F.A.: Role of the primate medial vestibular nucleus in long-term adaptive plasticity of the vestibulo-ocular reflex. J. Neurophysiol. 43: 1725-2745, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01090-04 LNP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of Central Nervous System Functional Anatomy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.15	1.8	1.45

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neuroanatomy research laboratory has been used this year for studies in the a) tracing of neuronal fiber connections in the brain, b) development of new in vitro autoradiography technique for localizing opiate and other peptide receptors in the brain, and c) localization of brain sites which are selectively involved in sleep and drug-induced sedation. The fiber-tracing technique, based on transport of tritiated amino acids, has been used to show by autoradiography a variety of laminar distributions of thalamic projections to cerebral cortex. These data provide structural evidence for mechanisms of thalamocortical integration. In addition, a new method for in vitro localization of brain receptors within slices of fresh-cut brain tissue has been developed. The technique provides a simple and general way of visualizing sites of action of a wide range of biologically active substances in any fresh brain tissue. Receptor sites for opiate derivatives and benzodiazapines are currently under investigation. A third application of the autoradiographic technique will be to visualize brain areas with special functional and metabolic characteristics. Labeled 2-deoxy glucose may reveal brain sites that have special metabolic properties during sleep states following various forms of experiences by rats.

I. Studies of CNS Connections

Objectives:

This project uses histochemical tracing techniques to map neuronal connections within the rat brain. Work nearly completed has dealt with the cortical projections of thalamic nuclei, which collectively may be called the "nonspecific" thalamus. The cell groups under investigation--the midline, intralaminar, parafascicular, ventromedial and ventrolateral nuclei--attracted interest over 30 years ago when electrophysiologists found that electrical stimulation of nonspecific thalamic sites in the cat could elicit widespread and synchronous cortical responses. The data further suggested that the pathways carrying these electrical events to the cortex were not only widely distributed, but were anatomically separate from the known thalamocortical pathways originating in the "specific" thalamic relay nuclei. Moreover, microelectrode depth recordings suggested that specific and nonspecific afferent fibers terminate in separate cortical layers. Unfortunately, however, the neuroanatomical tract tracing techniques, then based on degenerating axons, could find little evidence for nonspecific thalamocoratical projections terminating in wide areas and in unique laminar patterns. Consequently, the early workers abandoned their interest in the possibility that these mechanisms could in part underlie cortical and behavioral arousal phenomena, and even conscious integration. The present studies have provided the long-sought anatomical bases for the electrophysiological phenomena. They have also provided a new framework for categorizing thalamic nuclei based on the laminar distribution of their cortical projections.

Methods Employed:

The techniques of anterograde fiber tracing by tritiated amino acid autoradiography and location of cells of origin by retrograde axoplasmic transport of HRP, both developed in the last decade, have provided more sensitive and artifact-free data than the older degeneration methods. Moreover, the deposition of the tracer enzymes in the brain by microiontophoresis produces injection foci that are much smaller, yet more concentrated, than those produced by hydraulic pressure. The combination of these newly-developed methods has made possible the aforementioned research.

Most of the work done this year has been autoradiographic. Essential facilities were installed in August 1977 and have been in full operation since then. In addition, a technician routinely carries out careful and ultraclean histological steps necessary for producing good autoradiographic material.

The anterograde data can be corroborated by using the HRP technique to locate cells of origin of widespread thalamocortical projections. Placement of the retrograde marker into various cortical regions will label nonspecific thalamic neurons only if the histological procedure is maximally sensitive. Dr. Sandra Moon Edley has developed staining procedures for the visualization of the HRP reaction product using safe, non-carcinogenic substrates that offer maximal sensitivity and reliability. In addition we have developed new surgical procedures for the restricted delivery of the HRP tracer to only the superficial cortical layers. The histological and surgical successes give us hope that the technique will later be used for studies of the monkey brain, which presents additional histological problems because of its larger size.

Major Findings:

Previous work by the principal investigator utilizing the autoradiographic technique has shown a remarkable and unique projection to the most superficial layer of almost the entire neocortex originating from the ventromedial thalamic nucleus in the rat. Another widespread and hitherto unrecognized projection to the hippocampus and limbic cortices was found after placing a mixture of ^{3}H-leucine and ^{3}-proline into the nucleus reuniens of the thalamic midline group. Results from autoradiographic cases of isotope injection into intralaminar thalamic nuclei suggest another instance of widespread neocortical distribution but, in contrast to that of the ventromedial nucleus, intralaminar projections terminate in deep cortical layers. Some other thalamic inputs are distributed to two or even three layers, and experiments have determined that multiple and spaced laminations arise from adjacent thalamic cell groups. Further analyses will also provide the topography of the thalamocortical and thalamostriatal projections. Most of this information can be corroborated from HRP case material.

A major area of thalamus whose cortical connections were hitherto incompletely described includes the ventroanterior, ventrolateral and posterior nuclei. These nuclei, previously thought to project to the motor or sensory cortex, appear to have a much wider cortical distribution. Autoradiographic and HRP experiments have demonstrated widespread cortical projection terminating in superficial as well as in deeper layers. The unique patterns of lamination, as well as a fundamental shift in views of thalamocortical connectivity provided by these data, arouse our interest in these same systems in the monkey. Such experiments could provide a bridge between the anatomical work in the rat and some ongoing and planned electrophysiological/anatomical studies concerning the primate motor cortex.

II. Studies of CNS Functional Neuroanatomy. Neurochemical Investigations

Objectives:

Since the autumn of 1978 an extensive collaboration has been undertaken with Dr. Candace Pert in the Biological Psychiatry Branch of the NIMH. Our efforts have been aimed at developing new methods to localize specific brain regions, the sites of action of pharmacologically active drugs, and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of anatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and the fiber pathways and terminal distributions in any given region. This search for anatomical correlates is augmented by the use of immunocytochemical mapping techniques for selectively marking cells and axons containing the putative endogenous transmitters that are released onto the receptors. The assignment of a neurotransmitter to each of the identified CNS pathways is the ultimate goal of this type of effort.

Methods Employed:

We have successfully developed an *in vitro* autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue slices. Fresh cryostat-cut brain slices are securely attached to glass slides by a process of thaw-mounting and subsequent freeze-drying. Slides are then incubated in solutions containing a labeled ligand. Excess and nonspecifically bound ligand is washed off in cold buffered rinses, and the slides are lyophillized again. The sections are fixed in hot paraformaldehyde vapors under a vacuum, then dipped in radioactive-sensitive emulsion for autoradiography. Retention of the label on the section is monitored at each step by liquid scintillation counting.

Major Findings:

To our delight we found that the method of tissue fixation by dry, hot paraformaldehyde vapors also successfully "fixed" the tritiated ligand to the receptor. This technical breakthrough permits the use of traditional "wet" autoradiographic procedures for dipping slides into liquid nuclear track emulsion for the subsequent visualization of receptor localization. The combination of the *in vitro* labeling technique and "wet" autoradiography has resulted in opiate receptor localization whose resolution surpasses all previously published techniques. Through careful histological procedures we have also produced tissue of higher quality than previously attained. The same tissue is amenable to tract-tracing studies so that "double-labeling" experiments can be performed in the same animal. A paper in press describes our first efforts at surmising "opiate-ergic" tracts from the observed concordances between the autoradiographic visualization of tract terminations and receptor distributions in corresponding brain loci.

The method has applicability for all receptor types for which a drug or transmitter ligand has been identified. In addition, it can be used for cells grown in culture and on non-neural body tissues. Perhaps more exciting is the fact that it can be used on human post-mortem tissue and may open new doors into receptor-related disease states.

III. The CNS and Sleep

Objectives and Methods:

The uptake of tracer amounts of ^{14}C-labeled 2-deoxy-D-glucose (2DG) by brain tissue reflects glucose utilization of a particular region (see Project No. Z01 MH 01084-04 LNP for the method). A general lowering of brain metabolism by anesthetics occurs for most brain areas, but in this project it has been found that an anatomical system consisting of the habenula, interpenduncular nucleus and the habenulo-interpenduncular tract, shows relatively much greater labeling by ^{14}C-2DG during anesthesia. This demonstrates altered functional activity in an anatomically defined neuronal network including the interconnecting myelinated fiber tract.

Similar states of altered metabolism may occur during sleep. Current behavioral investigations done in collaboration with Dr. Martin Zatz in the Laboratory of Clinical Science in the NIMH are indicating that carbachol, a drug that mimics the action of the neurotransmitter acetylcholine, can produce profound and longterm changes in the activity and sleep-wakefulness cycle in rodents. The pharmacological and behavioral data can provide clues to the neurochemical mediators for circadian rhythms. Alterations in physiochemical states in the brain may be measured by two techniques already described in this report--one by the 2DG method and another by the in vitro autoradiography method. The applications of these techniques must await the outcome of the behavioral experiments designed to demonstrate the role of acetylcholine in sleep cycles. Alternatively these methods might be used to show some of the CNS functions during sleep as a consequence of prior experience. Such experiments are in the planning stage.

Significance to Biomedical Research and to the Program of the Institute, and Proposed Course:

The study of thalamocortical projections now published in overview form, shows more precisely the nature of thalamic terminations in the cerebral cortex. The newly developed neuroanatomical histochemical tracing methods, wherever applied, tend to reveal CNS connections in more detail and entirety than was previously demonstrated or imagined. In this case, they show that a traditional concept--that thalamocortical fibers terminate mainly in the intermediate layers--must be revised in light of the demonstration that the cell groups lying outside the main thalamic relay nuclei have unique forms of laminar afferentation. Just as the "wiring plan" of the cerebral cortex must include the lamination of specialized cortical efferent cells, now the various types of laminar inputs must be included for a better understanding of cortical function.

Likewise, the great spread of these nonspecific thalamic projections over many distinct cortical regions suggests an important form of neural integration. The emerging possibility that the widespread projections arise from thalamic nuclei which are themselves innervated by components of the extrapyramidal system (substantia nigra, cerebellum, corpus striatum) suggests some role of movement in states of awareness and arousal. The demonstration of similar neural connections in higher mammals is planned.

The study of the brain localization of radioactively labeled substances such as 2-deoxy-D-glucose, peptides and diazepam, is an emerging hybrid of recently developed histochemical and biochemical techniques. In each instance the knowledge to be gained permits new insights into functional neuroanatomy. Previous efforts to elucidate structure-function relationships were limited by the paucity of useful techniques. Autoradiographic localization of biologically and pharmacologically active substances provides a more powerful tool for observing how the central nervous system operates.

Publications:

Herkenham, M., and Nauta, W. J. H.: Efferent connections of the habenular nuclei in the rat. J. Comp. Neurol. 183: 19-48, 1979.

Herkenham, M.: Laminar organization of thalamic projections to the rat neocortex. Science 207: 532-535, 1980.

Taylor, D. P., Pert, C. B., and Herkenham, M.: Localization of specific high-affinity binding of vasoactive intestinal peptide (VIP) in rat brain. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function. Rockford, Ill., Pierce Chemical, 1979, pp. 917-920.

Pert, C. B., Taylor, D. P., Pert, A., Herkenham, M., and Kent, J. L.: Biochemical evidence for Type 1 and Type 2 opiate receptors. In Gross, E. and Meienhofer, J. (Eds.): Peptides: Structure and Biological Function. Rockford, Ill., Pierce Chemical, 1979, pp. 845-851.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01091-03 LNP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Motor Function in Patients with Neuropsychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jerome N. Sanes	Staff Fellow	LNP	NIMH
Others:	Edward V. Evarts	Chief	LNP	NIMH
	Donald B. Calne	Chief	ETB	NINCDS
	William K. Stuart	Computer Specialist	LNP	NIMH
	Heikki Teräväinen	Visiting Scientist	ETB	NINCDS
	Gopivinda Gopinathan	Clinical Associate	ETB	NINCDS
	Christopher D. Ward	Clinical Associate	ETB	NINCDS

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 2.60	PROFESSIONAL: 1.25	OTHER: 1.35

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this project are to (i) examine the respective contributions of central motor programming and afferent information in the control of arm movements in normal, parkinsonian, and cerebellar diseased humans and (ii) to study the psychomotor performance of patients with motor disorders due to central pathology. The first set of experiments uses the techniques of recording electromyographic activity and kinematics of limb position while subjects manually match a target display with either a skilled ballistic or slow velocity movement with a handle whose displacement controls a visual display. Movement amplitude, presence or absence of visual feedback of position, and disturbances of the subject's movements are independent variables. Preliminary observations indicate that large movements are performed relatively accurately independent of manipulation of the experimental variables but that accurate performance of small movements becomes increasingly dependent on visual information and the absence of limb disturbances during movement. The second set of studies examines a variety of psychomotor variables from patients with Parkinson's disease. Tests of movement speed, reaction time, and gait abilities have provided measures that correlated with clinically determined fluctuations in drug efficacy.

I. Central and Peripheral Control of Movement in Humans.

Project Description:

The importance of afferent information in the control of limb movements is controversial. Whereas it is clear that afferents exert potent physiological effects on spinal motoneurons and cells in supraspinal structures, it has been suggested that some of these afferents contribute little to the final positioning of a limb. A case in point is the observation that muscle spindle activity does not reflect muscle length during rapid movements of large amplitude, thereby casting doubt on a regulatory role for spindles at the end of movements. In addition, physical disturbances imposed during movements, that likely activate muscle spindles, do not appear to modify final limb positioning. It is the object of the present project to further examine the role of peripheral inputs in the control of limb movements. Both normal volunteers and patients with neurological disorders will be studied during performance of movements of varying sizes. Different types of limb disturbances will be imposed during the movements.

Methods:

Human subjects are trained to manipulate a handle that is attached to a servo-controlled torque motor while performing either supination-pronation forearm movements, extension-flexion of the wrist, or extension-flexion about the elbow joint. The handle's displacement causes movement of a visual display beam, position beam (PB). The subjects are required to displace the handle to match another display beam, target beam (TB), and when TB abruptly changes position subjects are instructed to rapidly initiate a movement intended to match PB with TB. Subjects are informed before the experiment to always perform the movement either as rapidily as possible (ballistic-type) or as accurately as possible (slow-type). For each of three or four movement sizes (1°, 3°, 10°, and 30°) subjects are given approximately 250 training trials. For the first 150 training trials visual feedback of the handle position is present at all times, but for the final 100 training trials the PB disappears upon movement initiaion on one-half the trials. Direction of movement and presence or absence of visual feedback are pseudo-randomly ordered. Movements are performed against no load or in the presence of assisting or opposing loads of variable force. After task acquistion, subjects perform each movement for at least 250 trials on several occasions. At this time the PB is present 50% of the time and, furthermore, ongoing movements are pseudo-randomly physically disturbed 16% of the time. The brief disturbance can be a physical stop, or either a ramp opposing or assisting movement. In the event of the latter two physical disturbances, the ramp moves the limb one-third of the total distance toward or away from the starting position. Electromyographic (EMG) activity is monitored via surface electrodes. Analog signals of position, velocity and force and EMG activity are recorded and analyzed offline by a PDP-11/40 computer. The analyses focus on the patterns of muscle activity and the difference between hand or arm and target position (movement error). Error is measured at two points during each movement:

(1) when velocity first reaches 0 after movement initiation and (2) the average position from 450-500 ms after the first measurement point.

Preliminary Findings:

Motor behavior of normal subjects and parkinsonian patients has been studied for this project. Presently the subjects have all been studied under no-load conditions when ballistic movements were performed. Several tentative findings have emerged:

(1) For both parkinsonian patients and normal humans the amount of relative error increases as movement size diminishes. Positional error when velocity is first zero following movement initiation is usually less than a mean of 10-15% for the 30° movement, but can increase to 40-50% for a 3° movement. At attainment of final position, error is less that 5% for the 30° movement but often greater than 15% for the 3° movement. The presence or absence of PB does not influence positional errors when velocity is first zero but does somewhat increase error at final positioning.

(2) In normal subjects limb perturbations at the onset of movement result in an increase of relative positional error compared to when no physical disturbances occur. This effect increases as movement size diminishes, so that, for example, error is increased to about 20% versus 10% with no disurbances for 30° movements error when velocity is first zero, and for 3° movements 60-80% versus 40-50% in the absence of perturbations. Additionally, contrary to earlier reports, the limb's final position deviates from the target position when the limb is perturbed. Thus, for large movements the final positional error can be either less (i.e., positional undershoot) or greater (i.e., positional overshoot) than the spatial value of the target; for small movements the final position is invariably greater than the target position following disturbance of the limb.

(3) When movements of patients with Parkinson's disease are briefly opposed, the arm position tends to undershoot the target position. Since patients with Parkinson's disease perform "ballistic" movements relatively slowly, and often in a hypermetric fashion, the results obtained from this patient population must be carefully studied.

Significance to Biomedical Research and to the Program of the Institute:

Additional clarification of how tactile and positional sensory information is utilized to control skilled motor activity is essential to the understanding of normal and abnormal motor behavior in humans. It is hoped that these results will contribute to the understanding of how movements are initiated and completed. Finally, these studies will potentially develop standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents.

Proposed Course:

The project described in the first half of this report has only recently been initiated. More than two years of experimentation will be required to validate and extend the preliminary findings in providing additional information on the control of limb movements in normal and neurologically diseased humans.

Publications:

None.

II. Objective Psychomotor Evaluation of Neuropsychiatric Patients

Project Description:

In last year's annual report we described a computerized system for obtaining objective, quantitative measures of motor function in patients with basal ganglia disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system was used to record and subsequently summarize data concerning motor functions in 18 patients participating in clinical trials of the experimental anti-parkinsonian drug, Lisuride. Thirty age-matched normal subjects were also tested, with the intention of establishing normative data for movement variables measured on our system.

Major Findings:

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects of normal subjects were observed.

Parkinsonian patients undergoing treatment with Lisuride were tested on a regular basis throughout the course of the buildup and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date has been permanently stored in computer files and is accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

Significance to Biomedical Research and the Program of the Institute:

Because of the modest cost and ease of administration, the tests that we have developed should prove to be of use in a wide variety of experimental applications that require computer recording and analyses of results.

Proposed Course:

This is an ongoing project to provide objective evaluation of motor function in patients with movement disorders. Presently the neuroevaluation system is being used to evaluate the relative efficacy of bromocriptine and Lisuride in treatment of Parkinson's disease. In addition to studying patients with parkinsonism, plans are underway to evaluate motor functions from a wide range of patients with motor disorders.

Publications:

Evarts, E.V., Teräväinen, H.T., Beuchert, D.E., and Calne, D.B.: Pathophysiology of motor performance in Parkinson's disease. In Fuxe, K. and Calne, D.B. (Eds.): Dopaminergic Ergot Derivatives and Motor Function. Oxford, Pergamon, 1979, pp. 45-59.

Teräväinen, H., Evarts, E.V., and Calne, D.: Effects of kinesthetic inputs on parkinsonian tremor. In Poirier, L. J., Sourkes, T. L., and Bedard, P.J. (Eds.): Advances in Neurology, The Extrapyramidal System and Its Disorders, vol. 24. New York, Raven, 1979, pp. 161-173.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01092-02 LNP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Corticocortical Connections in the Sensorimotor Cortex of the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Steven P. Wise	Staff Fellow	LNP	NIMH
Other:	Michael Weinrich	Surgeon	LNP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.80	2.0	.80

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of a broad neurophysiological and neuroanatomical investigation of the corticocortical and corticofugal connections within the somatic sensorimotor cortex and their role in the generation and control of primate motor behavior. One of the central questions concerning both the synaptic and general organization of the cerebral cortex is how inputs to a cortical region from a variety of cortical and subcortical sources combine to contribute to its output. We have chosen to concentrate on the precentral motor cortex (MI), known to project directly to spinal motor neurons, and those cortical areas in close functional association with MI, the supplementary motor cortex (MII); area 3a (a transitional region between MI and the postcentral somatic sensory cortex), and the premotor cortex (area 6). Three related approaches have been adopted for this project: (1) an analysis of the organization of peripheral inputs to MI, MII, and area 3a; (2) single unit studies of cortical areas which supply inputs to MI; and (3) an analysis of MI output neurons whose targets (e.g., spinal cord, basal ganglia, cortex) have been identified.

PHS-6040
(Rev. 10-76)

Objectives:

The inputs to the precentral motor cortex (MI) as well as its intrinsic neuronal circuitry, determine the output of MI projection neurons, including those projecting to the spinal cord. Since there are a finite number of sources of afferent input to MI, as determined by neuroanatomical methods, it is a feasible objective to investigate neuronal activity within these sources during motor behavior, especially those behaviors for which MI activity is best understood. As a result of these studies we hope to gain an understanding of afferent interaction in producing motor cortex output. Ultimately, an objective of this project is to examine the activity of neurons which project to and from MI identified by antidromic axonal stimulation.

A more general objective of this project is an improved understanding of the evolution, organization, and role in voluntary behavior of the entire motor cortex, a region which is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery, including MII and the premotor cortex.

Methods:

Training. Monkeys (Macaca mulatta or M. fascicularis) were conditioned to perform, with rigid head fixation, one of three tasks, depending on the experiments. (1) Monkeys were trained to make dorisflexion (upward) or plantarflexion (downward) movement of one foot upon receiving a visual cue. A motor coupled to a foot pedal delivered abrupt changes in torque. For each unit, its relationship with movements and peripheral stimuli was evalated using the usual correlational and statistical criteria. (2) Monkeys were adapted to having a foot attached to a pedal. A servo-controlled motor established the position of the pedal at any of five positions and passively moved between these positions at one of four angular velocities. The relationship of each unit to the velocity and amplitude of passive foot displacements was evaluated using peak and sustained neuronal firing frequency. (3) Monkeys were operantly conditioned to push one of four target keys when given a visual cue. The animal was required to press one target button while the next target button was illuminated, a delay period passed, and the next go signal occurred. Then the animal could respond by depressing the target key to receive a reward.

For all experiments, extracellular unit activity of neurons in the somatic sensorimotor fields (area 3a, MI, MII, the first somatic sensory cortex (SI), and the premotor cortex) was monitored, recorded on magnetic tape with an 8-channel tape recorder, and analyzed off-line with PDP-12 and PDP-11 computers. For the premotor cortex experiments (see 3 above), eye position was monitored using an electrooculogram (EOG), and attempted head movements were monitored using a strain gauge attached to the head restraint apparatus. The strain gauge and head restraint system, which allows the monkey's head to be oriented 20° to either left or right, was engineered by Mr. James Sullivan (Division of Research Services, Biomedical Engineering and Instrumentation Branch) following discussions with Drs. E. Evarts, M. Weinrich and S. Wise.

Following the recording procedures, small amounts (5-10 μCi) of [3H]-amino acid were injected into either the premotor cortex, SI or MII. By noting the ultimate distribution of radioactivity in the brain, the sites of termination of neurons in MII, SI or the premotor cortex can be determined by tissue autoradiography.

Preliminary Findings:

About 1300 units have been studied in four monkeys examined in this project to date; the examination of the input organization of the rostral sensorimotor cortex is nearing the completion of its initial phase and three reports of this work have been submitted for publication. Several findings and interpretations have been developed:

1. MII is virtually unresponsive to peripheral inputs, as compared with MI in the same monkey. This finding is somewhat surprising from a neuroanatomical perspective, since MII receives monosynaptic corticocortical input from most divisions of the somatic sensorimotor cortex. However, the lack of profound somatic sensory responsiveness of MII neurons supports the hypothesis that MII plays a role in centrally generated motor programs rather than movements regulated by peripheral feedback.

2. MI, which is very sensitive to peripheral inputs, can be divided into at least two regions, one rostral and the other caudal, on the basis of the broad submodality type of its peripheral inputs (e.g., skin receptors vs. muscle receptors). These results suggest that cutaneous inputs to MI, since they predominate in a discrete (caudal) part of MI, may be more important in cortical motor control than usually believed.

3. Both area 3a and MI neurons receive information concerning both the velocity and position of parts of a limb. These findings have enabled us to improve the current understanding of cerebral localization in this part of the cortex and the relationship of physiologically defined cortical regions with those defined by anatomical methods. Two of these points are most noteworthy: (a) microelectrode methods reveal that the boundary between MI and MII corresponds to the boundary between two anatomically defined parts of the agranular neocortex (termed areas 4 and 6) and (b) area 3a, the transitional field between the agranular and highly granular somatic sensorimotor cortex, appears to be, as it was originally defined (in the work of C. Vogt and O. Vogt, 1919), a discrete area characterized by a thin granular layer which receives inputs from muscle receptors concerning the velocity and, to a lesser extent, position of a limb.

The study of premotor cortex is in its initial phase, but one preliminary finding can be described. One population of units appears related to voluntary forelimb movements to particular points in space, and may be part of a mechanism which attains certain limb positions in space.

Significance to Biomedical Research and to the Program of the Institute:

Though it is often assumed in the neurological and neurobiological literature that different cortical fields relay specific information (e.g., sensory information) between each other via corticocortical connections, there is very little direct evidence which supports this conclusion. An alternative view is that corticocortical influences from a cortical region serve a number of functional roles which, in addition to information transfer (if it occurs at all), include more general influences on the activity levels or specific outputs of target cortical regions. Because of the potential significance of corticocortical connections in sensory information processing, cognition, and motor behavior of primates and other mammals, this neuronal system deserves a detailed examination.

Proposed Course: In order to further examine the function of cortical connections, we must acquire more knowledge about the input-output organization of the cortex. This project will be continued in the Laboratory of Neurophysiology with the collaboration of Drs. M. Weinrich, J. Donoghue, C. Fromm, and others.

Publications:

Coulter, J.D., Bowker, R.M., Wise, S.P., Murray, E.A., Castiglioni, A.J., and Westlund, K.N.: Cortical, tectal and medullary descending pathways to the cervical cord. In Granit, R. and Pompeiano, O. (Eds.): Reflex Control of Posture and Movement, Vol. 50, Progress in Brain Research. Amsterdam, Elsevier/North-Holland Biomedical Press, 1979, pp. 263-279.

Jones, E.G., Coulter, J.D., and Wise, S.P.: Commissural columns in the sensory-motor cortex of monkeys. J. Comp. Neurol. 188: 113-136, 1979.

Wise, S.P., Fleshman, J.W., and Jones, E.G.: Maturation of pyramidal cell form in relation to developing afferent and efferent connections of rat somatic sensory cortex. Neuroscience 4: 1275-1297, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01093-02 LNP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Role of Somatic Sensory Inputs in the Cerebral Control of Movements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Von Ayre Jennings	Staff Fellow	LNP	NIMH
Other:	Hugo Solis	Guest Worker	LNP	NIMH
	Yvon Lamour	Guest Worker	LNP	NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS: 3.35	PROFESSIONAL: 1.25	OTHER: 1.10

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a study of the role of sensory inputs in the cerebral control of motor behavior in the primate. It uses the technique of single neuron recording in behaving monkeys to directly compare the activity of neurons in primary motor cortex (MI) during the performance of learned arm movements with the activity of neurons in the parts of the somatic sensory (SI), parietal and frontal cortex which are reciprocally connected with MI. Two questions form the focus of our investigations. First, the possible modulation of peripheral inputs to the sensorimotor cortex during motor activity is being studied by recording SI and MI response to peripheral stimuli delivered during and before active movements, during different levels of steady state motor activity related to differences in the exerted force or length of the contracting muscles and during isometric versus isotonic contractions in which the animal controls either exerted force or arm position. Second, the relation of posterior parietal and frontal cortical areas to visually guided movements is being investigated by determining whether the neuronal activity in these areas reflect information concerning the visual cue to begin movement, as well as limb position or exerted force.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Peripheral inputs to MI during movement may influence motor activity in several ways. It is the object of the present project to examine two of these possibilities.

1. SI inputs may be rapidly relayed to motor cortex and thus form the incoming sensory limb of a transcortical reflex loop. MI responses consistent with this "servo" hypothesis have been observed when a moving or stationary limb encounters a sudden load. We therefore intend to examine the neuronal activity in those SI areas which are known to be densely and reciprocally connected to MI and determine if the salient input to MI might arise in these regions.

2. Peripheral inputs to MI may also affect motor performance via connections with the posterior parietal cortex (areas 5 and 7). A wealth of neurological, physiological and anatomical data suggests that these cortical areas participate in visually guided movements. One possible mechanism by which this may occur is by association of inputs from visual cortex about the position of a visual target with inputs from SI about the current position of the limbs. Outputs from this area could reach MI by known projections to frontal regions which, in turn, are connected to wide areas of motor cortex. Accordingly, we will examine the behavior of neurons in frontal and posterior parietal cortex which are related to aspects of limb mechanics, such as position or exerted force, and relevant visual stimuli such as a visual cue to begin movement.

Methods:

A monkey (*Macaca mulatta*) was trained to turn on the center lamp in a 15-lamp display by grasping a handle which was attached to a servo-controlled torque motor and either 1) exerting force in a pronating or supinating direction against the handle if it was held in position by the torque motor (isometric mode), or 2) making pronating or supinating movements of the handle if it was free to move (isotonic mode). The visual cue requesting a particular direction of exerted force or active movement consisted of a switch from the central lamp to one of the peripheral lamps in the display.

In the isotonic mode, alternating pronating and supinating movements between self-terminated end positions of 80° and 100° were made against a steady torque (.1 newton-meters) which was applied by the torque motor in either a pronating or supinating direction. Stimuli superimposed on these movements were step changes in handle position (position pulses, 50 msec, 8 - 10°) delivered at different phases of the task: 100 msec after the visual cue triggering the active movements (but before any active displacement), during active movement, and during the holding period between movements.

In the isometric mode, the handle position and force exerted by the monkey during steady holding were the same as in the isotonic mode except that the monkey controlled force rather than position. Thus, the monkey exerted a pronating or supinating torque of .1 newton-meters against the handle which was locked at a position of 100° or 80°. Stimuli consisted of 1) position pulses (50 msec, 8 - 10°) during steady holding and 2) passive limb movements of 20° induced by ramp displacemnts of the handle at a velocity similar to the velocity of active movements in the isotonic mode (150°/sec).

Following training of the monkey, a large chamber was attached to the skull which allowed single unit activity to be recorded in the extensive cortical regions encompassed by areas 6, 4, 3, 1, 2, 5 and 7 (i.e., the greater part of the sensorimotor and parietal regions of the cortex). Unit activity, handle position, exerted force and stimulus codes were recorded and analyzed off-line by a PDP-12 computer. For each unit analysis focused on the frequency and pattern of discharge at each steady state force level and handle position, and the change in frequency and pattern of discharge produced by position pulses, active or passive movements and active contractions.

After completion of recording procedures, the animal was perfused and the brain grossly and microscopically examined in order to correlate positions of electrode penetrations with areas of cortex.

Preliminary Findings:

Analysis of single unit activity recorded from 1612 neurons in both hemispheres of one monkey over a period of 14 months is now in progress. Thus far, five findings have emerged.

1. Of the SI neurons found to be sensitive to passive arm displacements, 30% showed a decreased response to isotonic position pulses delivered during active movements (as compared to the responses obtained during the holding period) and in some cases responses to pulses delivered prior to onset of active movements were also decreased. Thus, MI may exert control over its own peripheral inputs via cortico-cortical projections to SI.

2. The discharge of 25% of SI neurons was related to maintained wrist position. Each of these position sensitive neurons was reciprocally related to the direction of exerted force, i.e., they were more active for one force direction and less active for the other. Furthermore, the position and force sensitivities of most cells were congruent: cells more active with the wrist in a supinated position were also more active with supinator force and vice versa. Neurons of this type were found in three discrete zones identified as area 3a, area 2 and area 5. This indicates that neurons in cortical regions classically thought to subserve position sense are also sensitive to muscle tension and suggests that inputs to SI neurons from muscle receptors or central motor systems such as the motor cortex may play a role in perception of limb position.

3. Of the MI neurons with identified peripheral receptive fields, those classified as receiving cutaneous inputs from fingers, hand and arm were found to be concentrated in the bank of the central sulcus (MI/c) while neurons responding to non-cutaneous inputs were concentrated in more rostral regions (MI/r). MI/c neurons were characterized by a lack of sensitivity to maintained force or position and to the direction of active or passive movements while, in contrast, MI/r neurons were usually reciprocally related to the direction of active and passive movements and sensitive to maintained force and position. In addition, following the visual cue triggering active movements, the change in discharge frequency of MI/c units lagged that of MI/r units by an average of 61 msec and preceeded the first change in position by an average of only 42 msec. By showing that MI/c and MI/r neurons behave quite differently during motor behavior these results confirm previous suggestions that MI consists of at least two functional motor regions.

4. A population of neurons, termed VC cells, were found to have alterations in discharge frequency which were time-locked to the occurrence of a visual cue in contrast to the onset of activity of most other neurons which was better correlated with the beginning of movement. VC activity was always specific for a visual cue triggering a particular direction of contraction. These neurons were found in two distinct groups, one in area 7 and the other in a region posterior to the genu of the arcuate sulcus. VC neurons in both groups responded with a very short latency to a visual cue, 98 msec for those in area 7 and 133 msec for the postarcuate group. Since these two cortical regions are known to be anatomically connected, these results suggest that VC neurons may be links in a pathway by which motor commands reach MI following the occurrence of a visual stimuli which requires a motor response.

5. Most of the 1612 neurons displayed alterations in discharge frequency following a visual cue or active or passive movement; however, 314 cells (19%) showed changes in activity before either a passive displacement or visual cue or in some cases before both events. Such anticipatory activity before passive movements was seen only for neurons in MI whereas neurons with activity changes before visual cues were more evenly distributed in both MI and SI. These results indicate that, while neurons in wide regions of MI and SI are involved in the preparation for a motor event, only MI cells become active prior to an expected sensory event.

The above results provide evidence for distinct zones of cortical neurons which are characterized by particular types of peripheral input and sensitivities to force, position and direction of active and passive movements. These zones are independent of the classical motor and sensory cortex and instead take the form of two cutaneous core zones (MI/c and area 3b-1) surrounded by zones receiving deep inputs (area 3a, MI/r and area 2-5). In turn these regions are bounded anteriorly and posteriorly by functional zones related to visuomotor activities (postarcuate region and area 7).

Significance to Biomedical Research and the Program of the Institute:

Further clarification of how sensory information from the periphery is utilized to initiate and control skilled motor activity is essential to a better understanding of normal and abnormal movements in man. Though much is known about the involvement of motor cortex in motor control, more information is needed concerning the characteristics of sensory input to MI, the pathway taken by this input and its role in the initiation and control of motor behavior. As an important step towards this goal, the present project examines several cortical areas through which peripheral inputs could influence motor cortex output.

Proposed Course of Project:

Analysis of data from the first animal in this project is nearing completion and preparation for the next experiment is in progress. This experiment will utilize two independently moveable electrodes to study interactions between neurons in the cortical zones described above and, hopefully, provide further insights into the role of sensory inputs in the cerebral control of movements.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01335-10 SMRC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of Schizophrenia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Laboratory of Clinical Psychopharmacology

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CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The project summarizes the extensive studies with chronic schizophrenics including the development of a rating scale for measuring change over time the therapeutic testing of 5-hydroxytryptophan, tryptophan, alpha-methyl-para-tyrosine, pineal extract, physostigmine, lithium, baclofen, thyrotropin releasing hormone, naloxene, carbidopa, propanolol, as well as many biochemical determinations including monoamine oxidase.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To test existing and create new hypotheses relating to the possible organic basis of schizophrenia. This project attempts to synthesize psychological, physiological, and biochemical disciplines around the subject of schizophrenia.

Methods Employed:

All studies have been carried out in phenothiazine refractory chronic schizophrenics. The drugs were given and measurements made in a double-blind fashion.

Major Findings:

I. The Rating Scales

Nurses' Rating: Experience with the rating scale we had been using on the research wards to record clinical status indicated that it had many short-comings. We, therefore, undertook to design a more useful nursing scale that could yield quantitative estimates of the level of psychopathology of chronic schizophrenics. To be useful, the scale had to be as objective as possible and to be comprehensible to nursing staff trained to the level of nursing assistant.

Numerous conferences were held with the nursing staff to determine which psychiatric concepts were comprehended and could be inquired about on a rating form. Sample forms were devised and tested in the following manner: Every staff member on each shift would complete the rating on two patients. The results of this exercise were then subjected to statistical analysis in order to determine reliability and to find if, in fact, any meaningful consensus had been found as to the meaning of the questions. Different pairs of patients were used and the same patients were tested again to determine if reliability was consistent over time.

We have evolved a rating scale that appears usable by nursing staff trained to the nursing assistant level which will accurately reflect the daily clinical status of the patients. The new nurses' rating scale was tested extensively and various ambiguities eliminated. In an effort to shorten the scale, correlations were run between specific questions. These correlations did not hold consistently in each patient. Thus, no questions could be demonstrated to be expendable. Computor programs for processing the scale have been devised and a new optical scanning machine activated so that quick feedback can be gained from each patient.

The Inpatient Behavioral Rating Scale (IBRS) is an inventory designed to record daily nursing observations of mood and behavior of patients in a clinical setting. It consists of 26 items characterizing a broad range of psychopathology and is particularly suitable for use where change in mood and behavior is relevant to clinical investigation. It is formated on an optical scan sheet for ease of computer entry and analysis. In over a year of use on two clinical research wards, the IBRS has been found discriminating, reliable, and easy to use by the nursing staff.

Rating Scales: We have gained a years experience using the modified scaled version of the Brief Psychiatric Rating Scale. This scale, originally introduced by Overall and Gorham in 1962, has been extended and descriptive anchor points provided through a joint effort by Dr. Bigelow and Dr. Dennis Murphy. The scale as actually used by nursing staff has been shown capable of reflecting accurately the cycles in a patient with a periodic psychotic illness. Additionally the scale instructions have been shown to be remarkably reliable. This was established by having three psychiatrists of diverse backgrounds, naive to the scale, rate independently 15 patients in an interview setting. The intraclass correlation coefficients obtained from this exercise were quite impressive. We are in process of writing up this scale for publication. Since the original overall and Gorham BPRS has found extremely wide application, we anticipate that our scaled version will gain considerable popularity and application.

Premorbid Adjustment Scale: The premorbid rating scale has currently been used to rate 80 normals, 70 chronic schizophrenics, and 8 subjects diagnosed as having a schizoaffective disorder. The scale was found to successfully differentiate these three groups from each other. Differentiation on the basis of subdiagnosis was achieved only on the "late adolescence" and "adulthood" subscales for chronic paranoid versus chronic undifferentiated schizophrenics. Total premorbid adjustment score was significantly correlated with length of hospitalization. Further, the scale was found to differentiate between patients who had been chronically hospitalized for at least 7 years versus patients who were on an outpatient basis at the time of rating. The scale also differentiated patients who had had an insidious onset of schizophrenia from a group whose onset had been acute. In a study of CAT scans done on chronic schizophrenics, the premorbid adjustment scale distinguished between patients with normal versus abnormal CAT scans.

A study of chronic schizophrenics in India has recently been completed in which the premorbid scale was used. The scale was modified slightly for this purpose in order to more accurately reflect cultural differences. These data are now being evaluated.

Wechsler Adult Intelligence Scale: A previous study has shown that Parkinsonian patients treated with L-DOPA had decreased scores over time on the Comprehension subtest of the Wechsler Adult Intelligence Scale (WAIS), whereas scores on all other 10 subtests of the WAIS increased. It was hypothesized that if L-DOPA treatment, which increases dopamine activity in the brain, is directly related to an apparent deleterious effect on the WAIS Comprehension, then a drug such as alpha-methyl-para-tyrosine (AMPT), which decreases dopamine activity, might have an augmenting effect on this subtest. A therapeutic trial of AMPT in a group of chronic schizophrenic patients provided an opportunity to test this hypothesis. It was found that Comprehension scores improved significantly with AMPT. Other clinical rating instruments failed to show any changes.

Scores from 94 psychiatric and neurological patients on the 11 subtests of the WAIS and the Halstead-Reitan Category and Finger Tapping tests were used in a factor analysis. The Category Test had a low loading on the first verbal factor and a highly loading on the second, visuomotor factor; the Tapping Test had a high loading on a small fourth factor of manipulative speed. The finding with the Category Test suggests that it, contrary to the expectation of many of its users, does not distinguish an ability that is both separate from nonverbal intelligence and particularly sensitive to all types of brain pathology. It is concluded that visuomotor and verbal factor scores might be more efficient than conventional scores in studies of effects of brain damage.

Minnesota Multiphasic Personality Inventory: From an original sample of 375 college students and employees studied for platelet MAO activity, 66 subjects representing the lower and upper deciles were contacted for further study, including administration of the booklet form of the MMPI. Each subject was randomly assigned to one of two groups in which the low and high MAO subjects were balanced for sex. For women in Group A, a MAO scale discriminated low and high MAO subjects with 100% accuracy. For 18 men in Group A, a separate MAO scale discriminated with 94% accuracy. To cross-validate these results, the two scales were applied to another population; both discriminated low and high MAO women and men with a combined 97% accuracy. The thematic content of the two scales is discussed in the light of other reports on the psychological characteristics of low and high MAO subjects, including the apparent relationship between the scale content and clinical features of bipolar affective disorders.

Halstead-Reitan Neuropsychological Test Battery: Four of 17 chronic schizophrenic (or chronic schizoaffective) patients between the ages of 20 and 35, in partial remission, and living in the community were found to have mildly or moderately preminent sula, as disclosed by computerized tomography (CT scan). These four patients were matched for sex and age with four chronic schizophrenic (or schizoaffective) patients without sucal preminence. The Halstead-Reitan battery (HRB) of neuropsychological tests, including the Wechsler Adult Intelligence Scale (WAIS), was administered to these eight patients with the examiner blind to results of the CT scan. Each of the four patients with sulcal prominence considerably exceeded an established cutoff score for brain impairment on the HRB, whereas only one of the control schizophrenics performed in the impaired range and that just beyond the cutoff. The WAIS Verbal IQ, measured concurrently, was in the normal range for all subjects and similar for the two groups. These findings suggest that mild or moderate sulcal prominence on CT scans among young chronic schizophrenics is associated with impaired neuropsychological functioning.

A second study, including the HRB and WAIS, attempted to identify blindly those patients with evidence or morphological brain abnormalities on prior computed tomography (CT). The CT scan status of 12 of 15 (80%) patients was correctly identified solely on the basis of neuropsychological testing. These results supported our hypothesis that impairment on the HRB in chronic schizophrenic patients was associated with morphological abnormalities on the CT scan, and that the positive and negative CT scans of these patients could be predicted accurately.

Bender-Gestalt Test: The B-G was administered to 20 schizophrenic patients, 10 with tardive dyskinesia (TD) and 10 without tardive dyskinesia. Blind assessment of the patients' reproductions on two different occasions revealed a high test-retest reliability for the ratings. Significantly more TD patients (9/10) had organic signs than the non-TD patients (4/10).

II. Serotonin and Its Precursors

A. Behavioral: The study has now been underway for about five years. The first three patients were given alpha-methyl-5-hydroxytryptophan for one month, preceded and followed by one month placebo periods. The active drug made these patients worse.

The subsequent 18 patients were given L-5-hydroxytryptophan together with a peripheral decarboxylase inhibitor (MK-486). MK-486 blocks the conversion of 5HTP to serotonin outside the brain and because MK-486 does not enter the brain makes more 5HTP available for conversion to serotonin within the brain parenchyma. In addition, it is thought that MK-486 reduces 5HTP's side effects.

The patients were given placebo for one month, the active drugs for up to three months, and then a second placebo for one month. Behavioral ratings were done by nurses four times daily. The last 7 days of the highest active drug period were compared to the last 7 days of both placebo periods. For a change to have taken place, we required that the active drug period had to be higher or lower than both placebo periods ($p<0.05$, 2 tailed "t" test). By the criteria indicated, 3 patients have improved, 3 have become worse, and for 12, there was no change. It must be emphasized that these comparisons were made against placebo only and as yet, we have not determined how 5HTP compares to conventional drugs. What is clear is that these drug combinations chemically differentiate at least two kinds of schizophrenic patients; those who become better and those who become worse. Further efforts to determine if these chemical differentiations correlate with any behavioral distinctions are being made.

In order to determine if there are any additive effects of 5HTP and neuroleptics, we are now treating our patients with a constant dose of a neuroleptic, MK-486 and 5HTP (3-6 mg/day). The first three patients were given chlorpromazine as the neuroleptic and appeared to do well. The neuroleptic was then changed to haloperidol. Five male and two female chronic schizophrenics received a combination of haloperidol, L-5HTP and MK-486 in a double-blind, crossover study.

Patients were stabilized for at least one month on an optimal dose of haloperidol and an antiparkinson agent when necessary. After a month on haloperidol and placebo - 5HTP, patients began L-5HTP, 100 mg/24 hours, along with MK-486, 200 mg/24 hours. The intent was to follow a program of stepwise increments of L-5HTP dosage to achieve levels of 3 or more grams/24 hours as in the previous study, but this turned out to be impossible. Of the 7 patients, 3 became so activated on 100 mg/24 hrs of L-5HTP that they had to be removed from the protocol. All 7 patients showed some activation of illness in terms of their underlying pathology or character traits, but in 4 of the 7, these particular reactions subsided quickly, and permitted increased dosage of L-5HTP.

Preliminary data suggest that with each increment of L-5HTP, there was an increment of agitation or an exaggeration of existing pathology. There then followed in some patients an improved clinical status with better integration of thinking and more social interaction. This kind of improvement was vividly reported by 3 patients' families, who were, of course, unaware of the timing of the medication change.

These measurable clinical changes are striking, considering that such low doses of L-5HTP were used. We found earlier that without haloperidol, activation of symptoms occurred only when doses of L-5HTP of 2 to 3 grams per 24 hrs were reached. Tolerance to some side effects of L-5HTP developed in the patients during both studies. Transient parkinsonian side effects occurred in this study, whereas they did not at the much higher levels of 5HTP given in absence of haloperidol. It is clear that haloperidol in some way sensitized the central nervous system to exogenous L-5HTP.

Nine new chronic schizophrenic patients, three females and six males, participated in a second study of the clinical effectiveness of the drug combination 5HTP, chlorpromazine and MK-486. Doses up to 4 gm daily of 5HTP were given along with variable amounts of chlorpromazine. A common daily dose of chlorpromazine was 800 mg; however, patients received 200 mg MK-486/24 hrs. Although daily ratings of patients' attitudes and behavior are not yet available for analysis, no sustained clinical improvement was obvious in any of the patients. A preliminary impression is that 5HTP combined with chlorpromazine, like 5HTP and haloperidol, does elicit an increase in expression of affect. One patient in particular said repeatedly during active drug periods that she felt better, but no lasting change in paranoid ideas was seen. Apparently large doses of 5HTP in combination does not add much to therapy and may produce undesireable displays of irritability or uncontrolled affect in some chronic schizophrenic patients. An occasional patient may experience pleasurable affect on 5HTP without other marked change in clinical state.

Serotonin: L-5-hydroxytryptophan (L-5HTP) and the peripheral decarboxylase inhibitor carbidopa were administered to chronic schizophrenic patients in three separate experiments using a double-blind placebo-controlled crossover design. The three experiments were: (1) L-5HTP administration to 15 patients who had been withdrawn from all neuroleptic medication; (2) L-5HTP administration to seven patients maintained on haloperidol; (3) L-5HTP administration to nine patients maintained on chlorpromazine. Although the groups were diagnostically homogeneous, individual responses were highly variable. Considering each group as a whole, the only significant changes in rated psychosis consisted of an increase in the first group consequent to coming off neuroleptic medication and an increase in psychosis scores associated with adding L-5HTP to chlorpromazine. Neuroleptics apparently sensitize the central nervous system to the effects of L-5HTP loading. Acute exacerbations of psychosis induced by L-5HTP can be reversed by neuroleptics. Our experience does not give encouragement to the hypothesis that schizophrenic illnesses arise consequent to a functional deficit of serotonergic function that can be treated by giving a serotonin precursor in pharmacological quantities.

Whole blood serotonin concentrations were studied in 33 chronic schizophrenic patients previously having had CT brain scans and 23 healthy volunteers. The chronic schizophrenic patients had a mean serotonin significantly higher than the controls.

The patients were subdivided into those with abnormal CT scan findings (enlargement of cerebral ventricles and/or cerebral atrophy) and a group with normal CT scans. The patients with abnormal CT scans had significantly higher serotonin concentrations when compared to schizophrenics with normal CT scans as well as controls. The chronic schizophrenic patients with normal CT scans did not have significantly elevated serotonin concentrations compared to controls. Furthermore, ventricular size in the total patient group was significantly correlated to serotonin concentration.

Lilly 51641: (N-(2-chlorophenoxyethyl)-cyclopropylamine) a selective inhibitor of Type A monoamine oxidase (MAO) activity, was given for 9 to 73 days to eight inpatients with different primary psychiatric diagnoses. After two weeks treatment at daily doses exceeding 200 mg by mouth, this patient group exhibited greater than 90% mean reduction in plasma amine oxidase activity, which persisted for three weeks following discontinuation of the drug. Consistent reductions in platelet MAO were also noted, although the magnitude was less than 25% and such reductions persisted for less than one week following discontinuation of active drug. All 7 patients receiving active drug for longer than two weeks or at daily doses exceeding 200 mg by mouth exhibited significant and substantial mean increases in ratings of agitation or mania. The three female patients seemed especially vulnerable to such excitation during the seek prior to their menses. The only two patients with primary affective illness—one patient with unipolar and one with bipolar depression--had the lowest baseline platelet MAO activities, and showed dramatic antidepressant responses on the drug. In contrast, two schizo-affective patients showed either decreased agitation or increased depression during treatment with daily doses of 50 to 150 mg orally.

The one schizophrenic and one schizo-affective patient with highest baseline platelet MAO were the only two patients who exhibited an increase in psychosis on active drug.

Gross fragmentation of nocturnal sleep and daytime drowsiness was seen in all patients. The circadian rhythm in body temperature decreased in amplitude on the drug. All patients developed postural hypotension on this drug—a side effect which was managed adequately by dosage reduction and temporary bedrest.

B. Sleep Studies: Previously we found that chronic schizophrenic patients had a decreased number of rapid eye movements during REM sleep (REM density) and an increased number in NREM sleep. This situation is similar to one seen after the administration of parachlorophenylalanine (PCPA). PCPA decreases serotonin by inhibiting tryptophan hydroxylase. 5HTP reverses the sleep effects of PCPA-treated patients.

The sleep of 18 male, chronically hospitalized, schizophrenic patients was studied during prolonged 5HTP administration. This was compared to sleep studies of various lengths during the month-long placebo periods and immediately preceding and following 5HTP ingestion. During active drug administration, the peripheral decarboxylase inhibitor, MK-486, was also given. Seven of the 18 patients were studied at intermediate doses, 11 at high doses, and 7 during withdrawal from 5HTP.

At low dosages of 5HTP (2000mg) there was a significant increase in REM Latency (190 vs. 65), but no other changes in sleep. Total REM Sleep and Delta were significantly reduced on high dosages. Patients were agitated and sleep was poor during the first week of withdrawal; however, there was a significant increase in REM % and a decrease in REM Latency. During the second week of 5HTP withdrawal, all sleep measurements returned to baseline.

In addition, at the high dosages, the number of REM periods decreased compared to placebo (2.1 vs. 3.7, $p<.01$). Although the length of the first REM period was increased on high 5HTP dosages (43 vs. 18 min, $p<.02$), the time from onset of the first REM period to the second was unaltered.

The interpretation of the effects of 5HTP on sleep are complicated by such factors as dose, tolerance, and patient's behavioral response. Contrary to the hypothesis relating serotonin to sleep maintenance, total sleep on one side of the brain resulted in increased levels of 5HIAA in the dorsal raphe nucleus and striatum on the opposite side of the brain. There were no detectable changes of 5HIAA in the medial raphe, whose axons do not project to the striatum. Activation of the dorsal raphe was apparently by a retrograde process. The basal levels of adenylate cyclase were enhanced on the injected side of brain; however, there was no change of adenylate cyclase activity on the contralateral side of brain even though 5HIAA levels increased. The rise of 5HIAA on the contralateral side of brain was prevented by a hemisection caudal to the injection site. Our results suggest that it is possible to biochemically activate the metabolism of brain serotonin for days using regional injections of cholera toxin. Activation is not necessarily associated with long term changes of adenylate cyclase activity. Moreover, it appears that both caudate nuclei may be innervated by a single cell body or by separate cell bodies in ready communication.

Many psychiatric disorders are associated with abnormal metabolism of the biogenic amines in brain. Adenylate cyclase can be activated by the biogenic amines. Cholera toxin offers the opportunity for understanding the mechanisms for activating the cyclases of the amine containing neurons for prolonged periods.

The Habenula: Anatomical evidence suggests afferents to the raphe nuclei originating in the lateral habenula. The habenula nuclei have been proposed to play a unique role in funneling information from forebrain structures to the raphe. Supporting this hypothesis, recent electrophysiological experiments characterized neurons in this pathway as inhibiting the firing of raphe neurons. GABA has been postulated as the inhibitory transmitter. We have investigated in rats the short and long-term effects of habenula lesions on serotonin (5HT) turnover in the dorsal raphe and medial raphe and caudate nucleus, a dorsal raphe terminal area. In addition, GABA concentrations were determined in the dorsal raphe. Alteration in 5HIAA levels following lesions were used to estimate change in 5HT turnover. Both unilateral and bilateral habenula lesions produced statistically significant elevations in 5HIAA in the dorsal raphe when measured 16 hours and 7 days post lesion. There was no change in medial raphe or caudate nucleus 5HIAA. Dorsal raphe GABA concentration did not differ from control 7 days following biolateral habenula lesions. Although anatomical evidence suggests habenular projections to both dorsal and medial raphe, habenular lesions affect 5HT turnover only in the dorsal raphe. The lack of effect of habenular lesions on caudate nucleus 5HT turnover suggests that changes in cell body 5HT metabolism can occur without concomitant changes in terminal projections. These results support the presence of an inhibitory habenular raphe pathway but do not confirm GABA as the inhibitory transmitter.

Our finding of increased 5HT metabolism in the DRN following HBN lesions suggests that HBN-DRN afferents are inhibitory and complements previous findings of decreased 5HT neuronal firing in the DRN after HBN stimulation. The nature of the inhibitory input from the HBN is complex, EFFECT OF 5HTP ADMINISTRATION AND WITHDRAWAL ON MEAN SLEEP PARAMETERS

	P_1	5HTP (3600-5500mg)	P_1	5HTP (5600-6000mg)	P_1	Withdrawal
No. Subjects	7		11		7	
Total Sleep	318	274	335	273***	297	213
Stage II	223	209	219	18*	190	195**
Delta	6	0.04**	19	6***	28	11**
REM Latency	71	192	70	175***	91	19*
REM Time	78	52	86	69***	71	77
REM Density (Index)	1.4	1.6	1.5	1.6	1.5	1.7
REM %	23.2	19.4	24.3	25.2	21.9	36.2*

P1- Sleep during the month placebo immediately preceding drug
Withdrawal - Sleep during the first week following last drug administration
* $P < .05$ (Wilson matched-paired signed rank test).
** $P < .02$
***$P < .01$
Values are in minutes/night except for REM % and REM Density

decreased on 5HTP and contrary to the phasic-event suppression hypothesis, the REM density was not affected; however, REM density may not be the appropriate measurement of PGO spike density.

C. Biochemical: Cholera toxin is a non-reversible activator of adenylate cyclase from several tissues. Since adenylate cyclase is thought to be part of the receptor-complex in many neurons, we used cholera toxin to study whether adenylate cyclase was linked to regulatory receptors of serotonergic neurons. The rate of 5-HT metabolism was taken as an index of neuronal activation and we measured this metabolism in brain areas of rats injected with cholera toxin into specific brain regions.

Cholera toxin was injected into the raphe nuclei or caudate nucleus and serotonin metabolism was studied by high pressure liquid chromatography.

When cholera toxin was injected into the raphe nuclei, the metabolism of serotonin was increased in the striatal regions on both sides of the brain. Increased metabolism was manifested by an increase in the concentration of 5-hydroxyindoleacetic acid (5HIAA)for more than 72 hours. The change was a direct result of an increased rate of formation of serotonin rather than an inability of the brain to dispose of the acid. Injection of the toxin or the vehicle for the toxin into the brain ventricles was without effect on the serotonin system. Injection of toxin into the caudate nucleus possibly involving the depolarization of inhibition raphe interneurons by substance p-containing habenula-raphe afferents. Our results demonstrate that the habenula exerts physiological control of a specific raphe nucleus. Since both the DRN and MRN regulate different behaviors, the ability to selectively affect DRN metabolism via habenular manipulation should prove a useful tool in further studying the divergent function of the brainstem raphe nuclei. Furthermore, habenula lesions lead to decreased substance P levels in the dorsal but not median raphe within 24 hours. From this data, we propose a peptidergic innervation of the dorsal raphe nucleus by the habenula nuclei.

III. The Catecholamines

A. Behavioral

AMPT (Alpha-Methyl-Para-Tyrosine): Several lines of evidence suggest that certain types of schizophrenia may be associated with excessive dopamine activity. Psychotic symptoms in schizophrenics may be intensified by methylphenidate, which increases functional dopamine activity, and by L-DOPA, which is converted to dopamine. Amphetamine-induced psychosis in volunteers and in "speed freaks" is thought to be mediated by dopamine. The clinical efficacies of neuroleptics probably depend upon blockade of dopamine receptors. Alpha-methyl-para-tyrosine (AMPT) inhibits tyrosine hydroxylase, the rate limiting enzymatic step in dopamine synthesis. Although it should theoretically be clinically helpful in schizophrenics, two previous studies reported no effect when given alone. In a third study, however, Carlsson and his associates found that during treatment with AMPT, less thioridazine was required to treat schizophrenics. In this study, we report results of administering AMPT in a double-blind fashion to chronic schizophrenics who were concurrently treated with high doses of phenothiazines.

The subjects were 10 male chronic schizophrenics (age 19-35) who had been hospitalized for 2-15 years. Each received a constant dose of phenothiazines (average equivalent dose of chlorpromazine=800 mg/day). A four-week period of placebo administration preceded and followed by a three-week trial of AMPT (3 gm/day). Clinical effects were monitored by (a) the NIMH Nursing Rating Scale; (b) the BPRS administered by two psychiatrists weekly; and (c) the Comprehension Subscale of the WAIS before, during, and after AMPT administration.

As shown in the table, significant improvement was observed on the Comprehension Subscale ($p<.001$ compared with placebo periods) on AMPT. However, no improvement was noted in the schizophrenia items of the BPRS or the NIMH Nursing Rating Scale, except for one patient. Clinical impressions of three, nonblind psychiatrists suggested moderate but transient improvement during the first week of AMPT administration in 8 of 10 patients. Two patients developed symptoms on AMPT and one was withdrawn from the study. AMPT's effect on plasma levels of chlorpromazine (measured twice weekly) are presented in the table.

MEAN PLASMA CPZ (ng/ml)

Placebo	77.7	
AMPT	72.9	
		$p<0.01$
Placebo	109.0	

AMPT markedly increased prolactin secretion (see table).

MEAN PLASMA PROLACTIN (ng/ml)

Placebo	46.1	
		$p<0.002$
AMPT	111.6	
		$p<0.01$
Placebo	42.4	

MEAN (±SEM) SCORES ON FIVE CLINICAL OR PSYCHOLOGICAL VARIATIONS WITH AMPT

	Placebo	AMPT	Placebo	Analysis of Variance
Mean Psychosis Score (NIMH Inpatient Behavioral Rating Scale)	11.4±1.1	11.9±0.9	1.2±1.4	$X_r^2 = 4.20$, NS
Mean Schizophrenia Score (BPRS)	9.5±1.2	7.5±1.1	8.4±1.1	$X_r^2 = 5.85$, NS
Mean Comprehension Subtest Score (WAIS)	6.6±0.9	10.3±0.8	8.0±0.8	$X_r^2 = 11.56$ ($p<0.001$)
Mean Depression Score (NIMH Inpatient Behavioral Rating Scale)	7.0±0.5	8.0±0.4	7.1±0.5	$X_r^2 = 5.45$, NS
Mean Depression Score (BPRS)	5.9±1.3	4.8±1.2	4.8±1.3	$X_r^2 = 0.95$, NS

The results suggest that during a three-week trial with AMPT, an inhibitor of dopamine synthesis, subtle or no improvement in psychological functioning (judgment and comprehension) occurred in chronic schizophrenic patients on phenothiazines. Significant clinical improvement was not detected on the BPRS and NIMH Nursing Rating Scale.

In a second study, we administered AMPT (3 gm/day) for two-three months to seven chronic schizophrenic patients who were treated with thioridazine (800 mg/day). No major clinical benefits were documented in the second study, although serum prolactin appears to have been elevated, an indication of central dopaminergic blockade.

7-Hydroxy-Chlorpromazine: A number of biochemical, animal and human studies suggest that 7-hydroxy-chlorpromazine (7-OH CPZ) is an active metabolite of chlorpromazine (CPZ). This hypothesis was tested by administering 7-OH CPZ to four hospitalized chronic schizophrenic males (Research Diagnostic Criteria) using a double-blind crossover design.

Following a four-week placebo period, 7-OH CPZ was given for at least four weeks, three weeks of which were at 1000 mg/day. A second four-week placebo period was then followed by the administration of CPZ at the same dose and duration as 7-OH CPZ. Throughout the study, patients were evaluated twice/day with a nurse' rating scale and once/week with the BPRS. Serum prolactin levels were measured at least once/week. Data was evaluated by an analysis of variance and the Newman-Keul test.

Patients improved on 7-OH CPZ relative to placebo on the following NRS items: tension and uncooperativeness ($p<.01$); and blunted affect, hostility, excitement and arousal ($p<.05$). With regard to CPZ relative to placebo, improvement was demonstrated on tension and blunted affect on the NRS ($p<.05$). 7-OH CPZ was more effective than CPZ on uncooperativeness on the NRS ($p<.01$). On the BPRS, patients improved 7-OH CPZ relative to placebo on total score and emotional withdrawal ($p<.05$). Again, on the BPRS, CPZ was more effective than placebo on activation and anxiety-depression ($p<.05$). Finally, 7OH CPZ reduced emotional withdrawal more than CPZ ($p<.05$) as measured on the BPRS. Improvements on the NRS and the BPRS ranged from 25.5% to 50.9% for statistically significant items. Prolactin levels on 7-OH CPZ and CPZ were 33.1±4.5 ng/ml and 31.1+8.7 ng/ml, respectively. These were elevated by 119.2% and 84.0% over placebo levels of 15.1±4.5 ng/ml and 16.9±4.5 ng/ml, respectively ($p<.05$).

The above results clearly show that 7-OH CPZ is an active neuroleptic producing clinical improvement in chronic male schizophrenics. Moreover, it caused substantial increases in serum prolactin levels, a property of its metabolic precursor, CPZ, and numerous other antipsychotic dopamine blocking neuroleptics.

Propanolol: It has been reported that high doses of the β-adrenergic blocking agent propranolol can be therapeutic in schizophrenia. In an effort to confirm this finding, 11 young chronic schizophrenic patients have entered an ongoing double-blind crossover study of their clinical and metabolic response to this drug.

Subjects were patients on two research wards, who (1) were between age 18 and 45 years, (2) met Research Diagnostic Criteria for schizophrenia, (3) had had adequate trials of conventional neuroleptic treatment without achieving sufficient remission to enable living outside of a hospital setting, and (4) were able to consent or assent to participate in therapeutic trials. Patients were maintained on stable doses of neuroleptics throughout the trial. Treatment order, active-placebo or placebo-active, was determined randomly. Propranolol was given incrementally over a 5-wk period to a maximum of 1920 mg/24 hrs in two divided doses, unless the presence of side effects dictated a lower maximum. Patients were kept at their highest stable doses for 4 weeks and then tapered off propranolol over a further 4-wk period. Behavioral status was evaluated by the nursing staff who were blind to medication status using an expanded version of the Brief Psychiatric Rating Scale (BPRS). Behavioral ratings were analyzed as previously described.

Of the 11 patients entered in the protocol, 7 have completed a month of placebo and a month of stable dose of 1600 or 1920 mg of propranolol. One patient refused further participation after one week and a second patient had to be withdrawn because of postural hypotension. The results to date indicate the presence of modest changes in some ratings for each patient. No patient had a dramatic response, but the improvement in 2 patients seemed substantial to those who knew them.

Our clinical results thus far do not confirm earlier reports of marked benefit to treatment resistant patients, but the duration of treatment was longer in those trials. Further experience will be required to determine if there is a subgroup of patients who are responsive to treatment with propranolol. If such a group can be defined, it would be of great interest to discover the mechanism of action of propranolol in that group. Since propranolol is active both centrally and peripherally, and in addition has membrane stabilizing properties and antiserotonergic actions, there would be many possibilities.

IV. Physostigmine

Ten patients received oral physostigmine in varying degree in a controlled double-blind protocol attempting to replicate the earlier findings of Rosenthal that brief, but dramatic, clinical improvement could be observed during such treatment. None of the patients studied showed significant improvement. At this time, no further studies are contemplated.

V. Pineal Extract

Dr. Altschule reported in 1957 that injections of an aqueous extract of bovine pineal substance exerted a beneficial effect in chronic schizophrenia. A subsequent blind study on eight elderly, extremely chronic lobotomized schizophrenic women failed to show major therapeutic potential of the extract. We are testing the extract on a less chronically-ill population to see if there might be any merit to Altschule's earlier observations.

When the patients are clinically stable they are started blind on either a course of placebo or pineal extract. After 4-6 weeks on this medication, there is a crossover to the type material not already received. If the findings warrant, there is another shift of medication.

From the previous study done here using younger patients, it appears that there is a statistically significant but generally slight beneficial effect exerted by the extract on some patients. Only one patient can be said to have achieved a major remission associated with extract treatment. This finding has been confirmed in numerous crossover experiments.

A further three patients have been tried on pineal extract. Of the three, one responded with significant clinical improvement. Six months after completing the protocol, this patient was given a second trial of pineal extract vs. placebo injections and again showed significant clinical improvement coincident with extract administration.

The most pronounced responderhad been tried on enteric coated pineal tablets. Although an open study, the results were sufficiently encouraging to justify having more of these tablets made for further clinical trials, again in selected patients. The advantage of administering the pineal substance in this form is that dosage can be increased. The earlier studies with intramuscular preparations was handicapped by the inherent volume limitations imposed by that route of administration.

Two additional patients have been tried on enteric coated pineal powder tablets. One became markedly aroused and hostile; with the other there was no change. The one patient already shown experimentally to be a marked responder to the aqueous extract and thought in an open study to be responsive to the oral preparation went through a double-blind placebo substitution. The patient showed worsening of her psychosis (as judged by the nursing ratings) while on placebo and improvement when active medication was substituted. Further extensive clinical testing of pineal materials does not seem justified at this time. Efforts are being made using experimental animals to develop techniques for either predicting who the few pineal responsive patients might be or what might be a possible mechanism of action. Until a working hypothesis along these lines can be developed, it seems more practical to test clinically only those few patients who have a clinical profile quite similar to that of the most responsive patient.

We were unable to isolate a fraction of the pineal extract that would be significantly enriched with regard to inhibitory activity on the *in vitro* assay of indoleethylamine-N-methyl transferase. Further study with pineal and liver extracts indicated that the inhibition obtained from extracts of these tissues was quite non-specific.

VI. Lithium Carbonate with Other Neuroleptic Drugs

There is good documentation in the literature indicating that chlorpromazine is superior to lithium in acute schizophrenic illness. The superiority of chlorpromazine in controlling extreme mania also appears to be established. Publications bearing on the use of lithium in schizo-affective illness are less unanimous in their conclusions. All of the studies so far reported do not indicate a superiority for the use of lithium over chlorpromazine in patients diagnosed as schizo-affective. There are no studies of merit using both medications simultaneously. It is our personal experience that many clinicians are using lithium carbonate in combination with major tranquilizers in the treatment of schizophrenic and schizo-affective patients, despite the fact that there are no controlled experiments in the literature bearing on the efficacy of this combination.

This study has been expanded and is taking place mainly outside the William A. White Research wards. This has been done for a number of reasons, among them our interest in being able to study a greater number of patients on low-risk protocols such as this one. After several month's work, three wards in other services have been trained in the use of the rating instruments. In addition, one of the wards is in John Howard Division. This setting offers an excellent opportunity to study the effects of lithium ion on various aspects of aggression. The studies have been completed, and we are in the process of analyzing the data and preparing the manuscript for publication.

Lithium has been extraordinarily successful in the treatment of manic-depression. To compute the economic impact of that success on the United States, an estimate of the cost of care for manic-depression before lithium was introduced was compared with cost estimates after lithium. Economic gains in production were also calculated. Assumptions and exclusions err on the conservative side so that estimates, if inaccurate, are low. The use of lithium as a treatment for manic-depression has saved $2.88 billion in ten years and resulted in a $1.28 billion gain in production, or a conservative total of over $4 billion.

Computing lithium's impact on the cost of manic-depression required examining the cost of treating manic-depressive patients before and after the introduction of lithium. The task was not straightforward. It required (1) quantifying human life—we chose to do this through income, and (2) making a number of assumptions. Since little or no information was available on the number of manic-depressive patients in the United States, the number in treatment, their average length of stay in a hospital, and their basic pattern of treatment (including number of hospitalizations, outpatient visits and loss of worktime), the computations themselves rested on little hard data.

Our estimated impact of lithium on the cost of psychiatric care is impressive. Just as good nutrition and penicillin began to empty psychiatric wards of the victims of pellegra and syphillis and drastically reduce the costs of these diseases, lithium decreased the cost of manic-depression.

Lithium has a remarkable ability to ameliorate the mood swings of a manic-depressive. It is readily available and inexpensive. Furthermore, a response to lithium is widely thought to be diagnostic; many people who do respond to lithium would not previously have been diagnosed as manic-depressive and, perhaps, would not have received proper treatment.

Clearly, lithium has saved money. We estimate that in 10 years, from 1969-1979, America saved at least $2.88 billion in medical costs and gained $1.28 billion in production, a conservative total of around $4 billion as a consequence of the use of lithium to treat manic-depression. While our estimates are speculative and rest on limited data, they are—if inaccurate—extremely conservative. More solid calculations will become possible when more reliable information on numbers and diagnoses of people treated for psychiatric disorders are generated.

VII. GABA

A. Clinical

Baclofen (Lioresal): In the spring of 1975, a brief clinical report appeared in Lancet stating that 13 treatment resistant chronic schizophrenics were found to improve and require less conventional neuroleptic medication when placed on Baclofen. Since Baclofen, then thought to be a GABA agonist but now thought to be an antagonist of substance P, is a compound in no way similar to currently used neuroleptic agents, we have conducted a clinical trial.

In an attempt to replicate claims of baclofen's efficacy in treating schizophrenia baclofen was given to young chronic schizophrenic patients and plasma prolactin was monitored to study the mechanism of action of baclofen. Seven hospitalized male chronic schizophrenic patients were stabilized on clinically suboptimal doses of conventional neuroleptics (equivalent to 800 mg/day of chlorpromazine). Experience with these patients revealed that these doses were necessary for participation in the protocol and that larger doses resulted in less symptoms but not to the point that patients could function outside of the hospital. Baclofen was then added in doses of 80 to 120 mg/day for one month in a double-blind, crossover design. Twice weekly, fasting bloods were drawn 11 hours after the last neuroleptic and baclofen dose (into heparinized tubes which were then placed on ice). Plasma was removed and samples stored at -70 C until radioimmunoassay by the double antibody technique. The prolactin values during each period were stable for each patient as reflected by an intraclass correlation of .93 for the neuroleptic period and .87 for the baclofen period. For each patient, biweekly prolactins were meaned separately for the placebo and active periods and then compared by paired t-test.

No increase in plasma prolactin was found with the addition of baclofen (37.08 vs. 37.57 ng/ml, paired t-test, NS) to patients given sub-optimal doses of neuroleptics. There was no evident difference in response on 2 dose levels. Additionally, the patients did not clinically improve nor did they demonstrate change on rating scales.

Failure of baclofen to elicit an increase in plasma prolactin in these patients might be explained in several ways. Either the 80 to 120 mg/day dose (approximately 1 to 1.5 mg/ky) or one-month duration might be insufficient. In the rat, Meltzer and Fang found that only doses of 25 mg/kg and above produced increases in prolactin. Ravitz and Moore found that 5 and 10 mg/kg doses of baclofen slightly reduced prolactin, while 20 mg/kg markedly increased serum prolactin in the rat. They felt that the 20 mg/kg dose was anaesthetizing in the rat and the rise in prolactin was a non-specific stress-induced effect.

It is possible that prolactin in the patients was already maximally stimulated by the presence of neuroleptics and no further change would be expected by addition of baclofen. However, five of these seven patients, while similarly stabilized on neuroleptics (chlorpromazine equivalent to 800 mg/day), showed a dramatic rise in prolactin from 51 to 117 ng/ml (5=3.32, $p < .05$) when treated with three gms/day of AMPT.

Although baclofen has been used successfully to treat multiple sclerosis and other neurological disorders, its mechanism of action is still open to question. This study in humans demonstrates no alteration in dopamine metabolism as reflected by plasma prolactin concentrations.

B. Animal

Imidazoleacetic Acid: Chronic administration of neuroleptic drugs to animals results in a syndrome of behavioral hypersensitivity to dopaminergic agonists. It is thought that this is a manifestation of the development of postsynaptic supersensitivity to dopamine, induced by the dopamine-receptor blocking activity of these drugs. The phenomenon of postsynaptic denervation supersensitivity has long been known to be nonspecific in regard to particular neurotransmitters; this would suggest that chronic treatment of animals with dopamine agonists would also induce alterations in behavioral sensitivity to any other neurotransmitters for which receptors are located on the same neurons as dopamine receptors. There are gamma-aminobutyric acid (GABA) receptors in the striatum, and these seem to be intimately associated with dopamine-receptive neurons. It was therefore decided to test the possibility that alterations in the behavioral sensitivity to GABA agonists accompany the behavioral hypersensitivity to dopamine that is induced by chronic treatment with the dopamine-blocking neuroleptic haloperidol. It was found that the ability of the GABA receptor agonist imidazole-4-acetic acid (IMA) to reduce motor activity was lessened in animals that had been given haloperidol chronically added to the drinking water. When the time course of the behavior of the animals were examined, it was found that the activity of the control animals was high when first placed in a novel environment, but habituated gradually over the course of testing. Animals treated chronically with haloperidol, or injected with IMA, showed relatively small changes in total activity but essentially the same pattern of habituation. On the other hand, animals treated chronically with haloperidol and also injected with IMA showed almost a complete absence of habituation, in that their activity remained at or near its initial levels for the entire duration of a two-hour testing session. Acute injections of haloperidol had no such effect, and were simply additive with IMA in terms of decreasing behavioral activity. It is suggested that this alteration in responsivity to IMA, and presumably to central nervous system plays a role in the therapeutic response of schizophrenic patients to haloperidol.

VIII. Thyrotropin Releasing Hormone in Chronic Schizophrenia

Intravenous administration of synthetic thyrotropin releasing hormone (TRH) has been reported to lead to a prompt amelioration of schizophrenic symptoms in a pilot study involving four female patients. Drayson gave TRH to three patients with cyclical psychoses and failed to observe any clinical change. We gave TRH to three treatment resistant chronic schizophrenic males in an inpatient research setting in which all subjects were drug free for at least a month prior to study. Synthetic TRH, 600 g (Abbott 38579) or matching placebo, was administered by slow intravenous injection at 8 a.m. for five days followed by a two-day holiday and then crossover to the treatment not given. Order of treatment was random; neither the person giving the injection nor any of the clinical staff was aware of the nature of the substance being given. The patient's clinical status was estimated by the nurses' rating scale as well as by clinical observation by the resident psychiatrist. Particular attention was paid to each patient the first day of each series of injections because of the reported rapid onset of clinical change. Statistical comparison of active TRH vs. placebo was by t-test of the means for appropriate questions for the five days of each treatment period. The results are summarized in the Table.

The table shows that two of the three patients showed some worsening of their clinical condition, particularly patient 3. It is especially interesting to note that the depression score for these two patients were significantly greater during the week on TRH as compared to the week on placebo. This finding stands in contrast to earlier reports suggesting that TRH has antidepressant activity, and certainly TRH does not appear to be a useful drug for schizophrenia.

EFFECTS OF SYNTHETIC TRH ADMINISTRATION ON THE SYMPTOMS OF SCHIZOPHRENIA IN THREE CHRONIC SCHIZOPHRENIC PATIENTS. #: t-TEST

Patient# Treatment		Psychosis	Depression	Anxiety	Hallucinations & Delusions
	Placebo	3.20±.09	1.53±.16	2.80±.15	1.35±.62
1)	TRH	2.84±.25	3.18±.25	3.18±.22	2.20±.50
	P	NS	.05	NS	NS
	Placebo	3.47±.15	1.55±.30	2.68±.16	4.55±.28
2)	TRH	3.23±.13	1.23±.20	3.10±.21	4.23±.38
	P	NS	NS	NS	NS
	Placebo	3.34±.12	2.28±.12	2.93±.24	3.80±.19
3)	TRH	3.56±.14	3.53±.30	3.75±.12	5.43±.37
	P	NS	.005	.01	.005

IX. Formation of Tryptolines (Tetrahydro- -Carbolines) by Human Brain and Platelets

We have examined the products formed from tryptamine, N-methyltryptamine, and 5-hydroxytryptamine by an enzymatic preparation from either human brain or platelets, using 5-MTHF as a cofactor. In each case, we have demonstrated by TLC, co-crystallation, and gas chromatography/mass spectrometry that the reaction involves the addition of a carbon unit to the side-chain nitrogen, with subsequent cyclization to yield a tricyclic, tryptoline derivatives. These compounds have been found in plants, but have not previously been associated with mammalian systems. For both enzymatic preparations, the optimal pH is 6.5 and phosphate buffers of increasing strength enhance the reaction. For the brain enzyme, methylcobalamine, flavin adenine dinucleotide, and ascorbic acid each enhance the reaction, while cupric ion inhibits it. We have also done preliminary investigations on platelet enzyme activities in normal volunteers. We found marked diurnal changes and wide variability among individuals; however, male enzymatic activities were significantly lower than those for females. These studies demonstrate that 5-MTHF does not act as a methyl donor, as previously believed.

We have examined the properties and distribution of the enzymatic reaction in the human brain, using 5-MTHF as cofactor. With indoleamines, activity increases with either hydroxy or methyl substitution at the 5-position of the aromatic ring and decreases with the addition of methyl groups to the side-chain nitrogen. Both tryptopline and 5-hydroxytryptoline are substrates but 1-methyltryptoline is not. The enzyme has a similar Km for tryptamine and N-methyltryptamine and is inhibited by Cu^{++}, Ca^{++} and dithiothreitol. The enzymatic activity is unevenly distributed in the brain, with the highest activity in the hypothalamus and the lowest activity in the thalamus.

Acute ip injections of 5-hydroxytryptoline (5-HTLN) killed mice and rats between 12 and 48 hours post-injection with an LD_{50} dose of 320 to 350 mg/kg. Two weeks of daily injections of 200 mg/kg or 100 mg/kg 5-HTLN resulted in respective mortalities of 100% and 50%; surviving mice showed no changes in weight or gross appearance of brain, liver, lung, heart, spleen, kidney or adrenal. Neither acute nor chronic injections affected body weight or temperature except immediately before death where both dropped markedly.

In mice, one hour pretreatment with 200 mg/kg 5-HTLN ip, increased secobarbitol-induced "sleep time" (50 mg/kg, ip). At these doses, neither drug, alone, is lethal; yet, 50% of the animals treated with both drugs died 24-36 hours later. Intraventricularly, 10 µg of 5HTLN to rats decreased motor activity for 25 minutes following the injection.

Acute application of 150-300 µg of 5-HTLN to the isolated rat fundus strip contracts the muscle in a manner similar to 2-4 ng of 5-HT. When added to the superfusion medium, 1.0 µg of 5-HTLN causes a tonic increase in muscle tone without blocking 5-HT-induced contractions. Methysergide blocks these 5-HTLN effects. Preliminary studies suggest that 5-HTLN may act on serotonergic mechanism in the brain.

A case of folate-responsive psychosis which was associated with a defect in $N^5,^{10}$-methylenetetrahydrofolate reductase (methylene reductase) suggested the need to examine whether abnormally low activity of this enzyme might be of etiological significance in schizophrenia. We found that there was no significant difference in the platelet methylene reductase activity of chronic schizophrenics, compared to either hospitalized or nonhospitalized, age-matched control subjects (see table). Although it is possible that a larger survey might reveal a subpopulation of schizophrenics who are characterized by abnormal methylene reductase activity, this study suggests that chronic schizophrenia is not generally associated with such changes.

Comparison of Platelet Methylene Reductase Activity in Schizophrenic Patients and Control Subjects

	N	Age (Mean±SEM)	Methylene Reductase Activity (Mean±SEM)*	Range*	r**
Normal Controls	15	28.7+0.94	0.86+0.12	0.15+1.36	-0.31
Hospitalized Controls	16	27.7±1.25	0.72±0.13	0.15±1.36	-0.32
Schizophrenics	18	28.1+1.19	1.17+0.18	0.05+2.28	- .35
All Subjects	49	28.1±0.65	0.93±0.09	0.01±2.28	- .31+

*Expressed as nmole/hr/mg protein; ##Correlation of age to methylene reductase activity; +$p<0.05$

X. Sleep

The sleep of 13 drug free chronic male schizophrenics was studied during a one-month trial on chlorpromazine (CPZ) and compared with one-month placebo periods. Recordings were made only after subjects had been on the drug or placebo at least three weeks. Sleep latency and awake time was significantly less on CPZ while Stage II, Delta Sleep, Delta %, Non-REM activity, and REM Density were significantly increased. There were no significant changes in REM Latency REM time, REM %, Stage II %, and Non-REM %.

We investigated whether schizophrenics have a normal REM rebound following REM deprivation. In addition, since Cartwright et al. previously suggested that "good REM compensators" tend to be field independent, we related a measure of field independence (the portable rod and frame test) to degree of compensation.

Eight actively ill schizophrenic patients (E) (5F, 3M, mean age = 25) and eight non-psychotic control patients (C) (5F, 3M, mean age = 26) were partially REM deprived by the awakening method for two nights. Sleep on each of five Recovery nights (R_1-5) was compared with that of Baseline Sleep. All patients were unmedicated except for a single control patient who received chlorpromazine.

On R_1 mean change in REM compared with Baseline was +38 min for C and -2 min for E ($p<.01$); on R_2 it was +17 for C and -10 for E ($p<.05$). Mean change in REM during all Five Recovery nights was +14.5 min for C and +1 for E ($p<.05$).

Mean Baseline Sleep patterns (min) were similar in E and C patient group:

	Total Sleep	REM Sleep	REM Latency	REM %	Stage III + IV
E	378	93	72	25.0	37
C	390	99	78	25.5	32

Mean loss of REM was 71.8% for E and 72.8% for C. Controls had to be awakened significantly more frequently than E (35 awakenings compared with 28, $p<.05$).

A two-way multivariate analysis of variance—one factor being patient groups, the other being a repeated measure, linear trend analysis—revealed that C had significantly different sleep patterns than did E during Recovery ($p<.03$, $F=2.45$). The major source of difference was in REM sleep recovery ($p<.006$, $F=8.06$) but, surprisingly, Stage III + IV was also a significant contributor ($p<.02$, $F=6.13$). E tended to have elevated Stage III + IV on the first two Recovery nights and low and normal amounts on the last two except on the last Recovery night when Stage III + IV tended to be elevated, though not significantly.

REM rebounds during Recovery were correlated with increased Total Sleep ($r=.52$, $p<.01$). Nevertheless, after removing the effect of change in Total Sleep during Recovery by analysis of co-variance, the controls still had the normal pattern of REM rebound and the actively ill schizophrenic patients did not, i.e., the two groups differed in rate of REM decline over the five Recovery nights ($p<.03$).

Among four E and four C patients tested, there was a significant correlation between degree of REM rebound and field independence ($p<.05$, $rho=.79$). Mean change in REM during Recovery was 27 min for three field independent patients and -1.5 min among five field dependent subjects ($p=.036$, Mann-Whitney U-test).

These results suggest that actively ill schizophrenics differ from other psychiatric patients in failing to have a normal REM rebound. This difference was not attributable to differences in age, sex, baseline sleep, anxiety, or change in Total Sleep time during Recovery. REM compensation may also be correlated with field independence, though further research needs to be done to substantiate this finding before any conclusions may be drawn.

XI. Evoked Potentials and EEG

We studied the evoked potentials of 21 schizophrenics and 21 controls. They were matched for age and exposed to a 10-minute sequence of frequent and infrequent auditory stimuli. The amplitude of P_3, a response component of the evoked responses to infrequent stimuli, was much larger in the control group ($p < .0003$). Using a P_3 amplitude of 3.20 V in the first 2.5 minutes as a dividing point, only 7 of the 42 subjects were misclassified. This amplitude had a significant correlation of 0.48 with the patient's age at first hospitalization. The group P_2 amplitude to frequent stimuli was also much larger in the control group ($p < .003$). This amplitude has a significant correlation of -.41 with medication dosage in terms of equivalent amounts of chlorpromazine. Behavioral scales failed to correlate significantly with evoked response measures. Evoked response latencies did not distinguish the two groups.

A study is underway to develop a means of demonstrating whether or not schizophrenic patients have an unusual lateralization of brain functioning. Visual processing of verbal and non-verbal tasks is being tested to show if patients have recognizable differences from normal subjects. The tasks involve the use of a projection tachiostoscope and are being presented to normal subjects to establish normal patterns of response. Patients are being tested also, but inconsistent results have developed from limited trials. Should the theory behind this study prove workable, it could be applied to tests of various drug effects, or used to monitor the progress of treatment of patients in addition to providing information about a possible aspect of mental dysfunction.

XII. Brain Dopamine-β-Hydroxylase

Stein and Wise (1971) postulated that there might be a deficit in dopamine-β-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine, in schizophrenic patients.

Wise, Baden and Stein (1974) tested their hypothesis by collecting the brains of 18 chronic schizophrenics who had died in a state hospital and 12 persons without a history of psychiatric illness dying suddenly of heart attacks or accidents. The schizophrenics had decreased DBH activity ranging from 49 in the hippocampus, 58 in the diencephalon and 70 percent in pons-medulla. All these differences were statistically significant at $p < .05$. Because of these dramatic differences in the predicted direction, Wise et al. made a number of attempts to control for artifacts. After considering all these sources of artifacts, the large differences between schizophrenic and normal DBH activity remained.

We attempted to replicate their study. Specimens of autopsied brains from five chronic schizophrenic patients were obtained with the aid of the neuropathologist at Saint Elizabeths Hospital. Brain specimens from four chronic schizophrenics and nine controls were obtained by similar arrangement with the D.C. Medical Examiner's Office. Information about the subjects was obtained by interviews with family members, and hospital and police records. The control brains were obtained from periods without evidence of a psychiatric history, although one was from an individual with a police record and two were from heavy drinkers. The schizophrenics (7 males; 2 females) had a mean age of 49.2±6.3. Four died suddenly after traumatic suicides (in our experience, suicide is common among chronic schizophrenics), three from cardiac arrests, one from pulmonary aspiration, and one suddenly from pulmonary edema. The controls (all male) had a mean age of 42.3±4.5. Six died suddenly of trauma and three from cardiac arrests.

The periaqueductal pons-mesencephalon, hypothalamus and hippocampus were dissected out at autopsy and immediately placed on dry ice and subsequently stored at -80°C for up to one year. Wise et al. (1974) found DBH activity stable for one year at -15°C. There was no difference in mean storage time for the schizophrenics and controls. All DBH assays were performed by a staff member unaware of whether the samples were from schizophrenics or controls.

There was no significant difference in DBH activity in any area between the two groups. While the differences were not statistically different, DBH activity mean for the schizophrenic groups ranged between 77 and 89 percent of controls. Although seven of nine of our patients were taking phenothiazines at the time of death, Wise et al. (1974) indicate that this is probably not a cause of their DBH differences. Rats who had been given chlorpromazine (20 mg/kg 24 hour) for 12 weeks had a small increase in DBH activity. Although the patients in our study were taking a number of non-neuroleptic drugs, there was significant ($p<0.05$) negative correlations between the daily dosage of chlorpromazine or chlorpromazine equivalent and the DBH activity in the hypothalamus ($r=-.60$) and pons ($r=-.65$). This could mean that in the brains of schizophrenics, neuroleptics do decrease DBH activity, or possibly that there is a negative correlation between clinical pathology (as determined by the need for higher dosages) and DBH activity.

Wise et al. (1974) also attempted to determine the effects of post-mortem intervals on brain DBH activity (after a person dies, it may take several hours for that person to reach the morgue). Simulating this situation, rats that were killed and allowed to remain three hours at room temperature prior to assay had DBH activity reduced by 27%. In a similar experiment performed in our Laboratory over a six-hour period, there was 15% decrease in activity. Since Wise et al. (1974) human brain post-mortem intervals were "several hours" and ours a mean of 4.2 for the controls and 3.0 hours for the schizophrenics, this does not seem to be an important factor, except possibly for one control who was not brought to the morgue for 27 hours. Since he had the lowest DBH activity of any of the subjects, the DBH activities were examined for the controls with and without this subject's values. The exclusion of this value did not change the failure to reach statistical significance. Two subjects with death-to-morgue times of seven and 11 hours had

DBH activities about their group means. One subject with a 3.5 hour time lag was low. All other subjects had intervals of about one hour.

Wise et al. (1974) also compared the effects on brain DBH activity of the time from which the subject reached the morgue to the time of autopsy. They did this by storage of rat brains at $4^{\circ}C$ for up to three days and found a decrease in DBH activity of 18%. Their subjects' brains, however, ranged from one day to eight days at this temperature. Because of this, they matched time intervals for controls and schizophrenics and still found the DBH activity of the schizophrenics lower. Our controls were in the morgue for a mean of 9.7±1.9 hours, while the patients were there for 35.6+7.9 hours, which is statistically significant ($t=2.73$, $p<.02$). Furthermore, there are significant negative correlations between the time in the morgue to autopsy and DBH activity in the hippocampus ($r=-.53$, $p<.05$) and hypothalamus ($r=-.49$, $p<.05$) while that for the pons ($r=-.46$) was not significant. This suggests that the uneven distribution between the two groups in morgue time may be responsible for the small non-significant differences in DBH activity in our samples. Since the Wise, Baden and Stein brains came from two sources, it is possible some of their group differences may be due to different storage techniques.

We measured DBH activity both under maximal conditions as well as under non-maximal conditions, but did not find significant differences among patient groups and normal controls by enzyme procedure.

Differences in population characteristics such as race and sex is suggested by the trend in blacks to have lower DBH activities and females to have higher activities than males. The relationship of age to DBH enzyme activity is not known but may also be a contributing factor and should be further explored.

Previous reports have not determined DBH activity in various subtypes of schizophrenia. Although we found no significant differences among the subtypes and normals, the chronic undifferentiated group with paranoid features had a trend toward lowered DBH activity ($p<.2$ and $p<.13$). Further investigation of this subtype appears warrented.

One way analysis of variance shows no significant difference between control and schizophrenic subpopulations. The chronic undifferentiated schizophrenics with paranoid features had a somewhat lower mean DBH than the other patient groups or normal controls, although this does not reach statistical significance. The normal control group had a mean DBH of 37.28±26.00.

In addition, DBH and monoamine oxidase activities were studied in 13 schizophrenic patients and available first degree relatives. There was no association of low monoamine oxidase and low DBH activities within the schizophrenic families.

In summary, in our investigations, we have been unable to confirm the findings of low plasma DBH activity in chronic schizophrenic patients or the association of low DBH and MAO activity in pedigrees of schizophrenic probands.

XIII. Dopamine Sensitive Adenylyl Cyclase

Striatal adenylyl cyclase activity from autopsied human brain is selectively stimulated by low concentrations of dopamine. Under the experimental conditions used, dopamine was about three times more potent than norepinephrine. Histamine and serotonin were ineffective. This stimulation by dopamine was competitively inhibited by haloperidol. There was no difference between nine of the autopsied brains (head of caudate) of the control subjects without a psychiatric history and seven patients with chronic schizophrenia.

IX. Biogenic Amines, Their Precursor Amino Acids and Their Metabolites

The role of biogenic amines in mental illness may include amines other than serotonin and catecholamines. In the present state of our understanding, little information is available on these amines. To bridge this gap, we have undertaken the task to develop comprehensive and sensitive mass fragmentographic methods to measure many of the known biogenic amines, their amino acid precursors and their metabolites.

Our initial goal is to apply the method developed to measure the concentrations of the various amines and their derivatives in plasma, cerebrospinal fluid (CSF) and brain tissues. After establishing the normal ranges of these substances in biological materials, we hope to carry out several clinical and pharmacological studies in order to probe further the possible role of biogenic amines in mental illness.

The principal technique employed is mass fragmentography. To enhance the specificity of the method, selective gas chromatographic columns are used. These columns were chosen after extensive preliminary trials with various stationary phases and with different lengths of columns.

The biogenic amines, their amino acid precursors and their metabolites are partially separated and concentrated by either extraction into organic solvents or by thin layer or column chromatography. They are next dried down, derivatized to their appropriate pentafluoropropionate or trifluoroacetate derivatives and then injected into the GC column.

To correct for variations in processing the samples, internal deuterated isomers of the compounds of interest were synthesized, run through the whole procedure and then used to correct for any inconsistency.

The metabolites derived from tyramine, octopamine, dopamine and norepinephrine were extensively studied in the rat brain, lumbar and ventricular CSF. Mass fragmentography was carried out on the methyl ester/pentafluoroprionate (ME/PFP) derivatives of the acidic and the PFP derivatives of the alcoholic metabolites, employing an 8 ft. 3% SE 52 column.

In nine patients with ventricular-atrial shunts (5 males, 4 females, age infancy to 65), ventricular metabolite concentrations (ng/ml, mean ± S.E.M.) were: p-hydroxyphenylacetic acid (22.4±3.6), p-hydroxymandelic acid (7.1±1), vanillylmandelic acid (3.1±0.3), dihydroxyphenylacetic acid (9.7±1.6), homovanillic acid (154±14.4), free 3-methoxy-4-hydroxyphenylglycol (MHPG) (11.7 ±1), conjugated MHPG (4.6±1.3), free p-hydroxyphenylethanol (PHPE) (12.3±1.7) conjugated PHPE (less than 2 ng/ml). The levels of the acidic metabolites in the lumbar CSF were all significantly lower than those in the ventricular CSF. The alcoholic metabolites on the other hand were not significantly different. P-hydroxyphenylglycol was also semi-quantitatively measured and found to be present almost completely in the free form.

Lumbar cerebrospinal fluid (CSF) of schizophrenic patients was studied.

Treatment with oral probenecid (100 mg/kg) increased the concentrations of p-hydroxyphenylacetic, 3,4-dihydroxyphenylacetic and homovanillic acid by over three-folds compared to the baseline levels. The concentration of p-hydroxymandelic acid was also increased, but only moderately compared to the increase of homovanillic acid. Vanilmandelic acid showed a tendency towards being elevated by probenecid, but in our study, the elevation was not statistically significant, probably due to the large variance between subjects.

P-hydroxyphenylacetic, p-hydroxymandelic, homovanillic, 3,4-dihydroxyphenylacetic, vanilmandelic acids and 3-methoxy-4-hydroxyphenylglycol were measured in control, acutely treated and ethanol-dependent rats.

Ethanol did not cause a shift from an oxidative to a reductive pathway in the metabolism of amines as is well established in the periphery. Ethanol was found to have no effect on the transport of the acidic metabolites across the blood brain barrier.

Metabolites derived from dopamine and norepinephrine were significantly elevated in both acutely and chronically treated rats while blood ethanol levels were high. During withdrawal (no ethanol was present in blood) only norepinephrine metabolites remained elevated in rats rendered dependent on ethanol.

The concentration of VMA and MHPG in whole rat brain is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about 1 and 8 percent. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of formation of VMA is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA and MHPG is 1.4 and 0.38 hr-1, respectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norepinephrine metabolism in brain.

The concentration of the free, glucuronide and the sulfate conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in human plasma, cerebrospinal fluid (CSF) and brain by mass fragmentography.

All three forms of MHPG were detected in the media analyzed. Free MHPG was found to be the predominant form in both the brain and the CSF. The sulfate conjugate of MHPG constitutes about 15% of the total MHPG in the CSF while in the brain the percentage varies between 30% in the hypothalamus and cortex and 80% in the substantia nigra.

The concentration of the glucuronide conjugate of MHPG measured in the brain and CSF represents about 5% of the total MHPG concentration.

In the plasma free MHPG and its glucuronide and sulfate, conjugates are present in about equal concentrations.

The relative concentrations of the three forms of MHPG measured in plasma, CSF and brain were compared with their concentrations in the urine from previously published results. From this comparison the diagnostic significance of each of the three forms of MHPG in the clinical assessment of central norepinephrine metabolism is discussed.

Human plasma concentrations of the major acidic metabolites of m- and p-tyramine, octopamine, dopamine, epinephrine and norepinephrine was measured. The free and conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were also measured. The means and ranges of concentrations of the acid metabolites were comparable except for m-hydroxyphenylacetic acid. Free, sulfate, and glucuronide conjugated MHPG were also found in about equal concentrations.

In a collaborative study with Dr. Norton Neff of the Laboratory of Preclinical Pharmacology, dopamine metabolism was studied in various regions of rat brain following the decline of 3,4-hydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) from brain after treatment with pargyline, or from the accumulation of the acids after treatment with probenecid. The decline of DOPAC and HVA after pargyline treatment appeared exponential in all regions of brain studied with half-lives of about 13 min for HVA and 6.5 min for DOPAC. DOPAC was the major metabolite of dopamine, with various brain regions producing between 2 to 5 times more DOPAC than HVA. HVA accumulated after treatment with probenecid but the accumulation in one hour did not account for all of the HVA apparently eliminated from brain. DOPAC accumulated in some regions of brain (medulla, hypothalamus, and midbrain) and not in others (cerebellum, cortex, striatum, and hippocampus) after probenecid treatment. We conclude that dopamine metabolism is not uniform in brain and that the accumulation of DOPAC and HVA in brain after probenecid treatment only accounts for a minor action of the dopamine formed in brain.

Also in collaboration with the Laboratory of Preclinical Pharmacology a study was undertaken to measure phenylalanine hydroxylase activity _in vivo_ by measuring ^{18}O-tyrosine in plasma after exposing rats to ^{18}O. We were able to show that ^{18}O is incorporated in tyrosine _in vivo_ and that the presence of ^{18}O-tyrosine in plasma is a relative measure of phenylalanine hydroxylase activity. Treatment with p-chlorophenylalanine decreases the ^{18}O incorporation into tyrosine. Because of the simplicity of the procedure and its easy adaptability to human studies, the incorporation of ^{18}O into endogenous constituents might serve as a useful diagnostic procedure for some metabolic disorders, for example, phenylketonuria. These results also indicate that labelling with ^{18}O cannot be used to measure the turnover rate of brain catecholamines as previously proposed.

Sympathetic Ganglion: We also studied dopamine metabolism and function in the rat superior cervical ganglion. Trans-synaptic modulation of dopamine metabolism in rat superior cervical ganglion was evaluated by contrasting normal and surgically decentralized ganglions. Dopamine metabolism was assessed from the decline of DOPAC after administering pargyline and from the concentration of dopamine and DOPAC after treatment with drugs. The concentrations of dopamine, HVA and DOPAC were about 19, 2.7 and 51 pmol/ganglion, respectively. DOPAC was the major metabolite of dopamine and its calculated rate of formation was 5.3 pmol/ganglion/min, which is approximately 300 nmol/g/hr. This is about 15 times faster than the rate of formation of dopamine reported for striatum. The rapid formation of dopamine in ganglion was supported by the finding of a rapid decline of dopamine after ℓ -methyltyrosine treatment. In the same rat decentralization of the ganglion reduced the rate of formation of DOPAC, but increased DOPAC formation above normal in the intact sham decentralized ganglion suggesting that a feedback system may modulate dopamine metabolism. Treatment with carbachol alone or in combination with atropine of hexamethonium revealed that dopamine metabolism is modulated by nicotinic and muscarinic mechanisms. HVA and DOPAC accumulated in ganglion after treatment with probenecid indicating that an acid transport system is probably responsible for removing these materials from the ganglion. The similarities between the dopaminergic system of the ganglion and of the brain and the rapid rate of dopamine metabolism in the ganglion supports the notion that dopamine might serve as a transmitter or modulator of synaptic events with the ganglion.

During the last 12 months, our work on the sympathetic ganglion was aimed towards achieving two goals: the first was to gain more knowledge on how preganglionic muscarinic and nicotinic cholinergic inputs affect the small intensely fluorescence (SIF) cells with regard to DA turnover and metabolism. The second goal was to characterize pharmacologically the catecholamine receptors present in the sympatheic ganglion and to determine how these receptors regulate catecholamine turnover and synthesis. The ganglion studies were: superior cervical (SCG) and celiac (CG).

The SIF cells were found to be mostly stimulated by muscarinic preganglionic nerves. For example, decentralization lowers DA turnover as reflected by a significant decrease in DOPAC concentration in the SCG. Administration of carbachol or oxotremorine (muscarinic agonists) increased DOPAC while atropine decreased DOPAC in both SCG and CG. Hexamethonium or arecoline (nicotinic agonists) have very little effects on DOPAC concentrations. However, nicotinic receptors appear to have some inhibitory effects on the muscarinic stimulation of the SIF cells.

The concentrations of DA, NE and DOPAC in the ganglia were critically evaluated after treatment with the following basic drugs: reserpine, haloperidol, apomorphine and phenoxybenzamine.

Reserpine significantly decreased NE and increased DOPAC, but had no effect on DA concentrations in the sympathetic ganglia.

Apomorphine, contrary to what we expected, had no effect on catecholamine and slightly increased DOPAC (we expected a decrease). Pretreatment with apomorphine, however, antagonizes the effects of reserpine, thus indicating that reserpine and apomorphine exert their influences via a common receptor.

Haloperidol increased DOPAC in the CG but not in the SCG. It had no effect on the NE and DA concentrations. These results suggest some differences between the DA receptors in the SCG and the CG.

Phenoxybenzamine had no effect on DA, NE, or DOPAC in the ganglia.

XV. N,N-Dimethyltryptamine (DMT) and Phenylethylamine (PEA)

A. Behavioral

Two separate drug-induced behaviors in the rat that satisfy criteria which must be met for animal models of schizophrenia have been described. N,N-Dimethyltryptamine (DMT) and β-Phenylethylamine (PEA) were the two drugs studied because evidence suggests that they are viable candidates in the pathogenesis of schizophrenia. Both are endogenous compounds, found in human tissue. Both may be differentially elevated in psychiatric patients. When given to man, DMT produces psychological disturbance and amphetamine (almost structurally identical to PEA) can produce a syndrome resembling paranoid psychosis. Instead of trying to find an animal equivalent of schizophrenia, we studied the behavioral effects of these compounds in rats and applied to these behaviors criteria derived from the clinical condition of schizophrenia. Both DMT and PEA produced dose response disruption of a well-trained conditioned avoidance response in the shuttlebox and PEA, with monoamine oxidase inhibition by pargyline, induced dose response stereotypy, associated with increased motor activity. The criteria we tested were alterations of the drug induced behaviors by acute or chronic neuroleptic pretreatment and tolerance development. Neuroleptics, which are efficacious in the treatment of schizophrenia, reduced DMT disruption and PEA stereotypy in a dose response relationship. In the case of DMT, disruption was attenuated to a degree directly related to length of neuroleptic pretreatment, consistent with long-term antipsychotic efficacy of neuroleptics. Pretreatment with dopamine antagonists and agonists suggests that the mechanism involved in DMT blockade may be through denervation supersensitivity of dopamine receptors. In the case of PEA, attenuation of stereotyped behavior paralleled the clinical potency of neuroleptics on a mg/kg basis and in terms of relative ratios. Complete tolerance did not develop to DMT disruption or PEA stereotypy, consistent with the chronic course of schizophrenia. However, rapid tolerance develops to PEA disruption of shuttlebox avoidance behavior if doses subthreshold on PEA induced stereotypy. The drug induced behavioral models reported here are of particular interest because they involve endogenous compounds implicated in the pathogenesis of schizophrenia and there is some degree of pharmacological isomorphism between these models and the clinical condition of schizophrenia.

B. Clinical

The use of GLC-MS in psychiatric disorders is particularly important as endogenous compounds of interest are frequently present in low concentrations and specificity is of considerable concern. This is particularly true for dimethyl tryptamine (DMT) and its potential role in schizophrenia. The indoleamine DMT is a known psycotomimetic agent, and has been reported to be present in human blood and urine. Moreover, an enzyme system capable of forming DMT has been demonstrated to be present in man.

Using this assay, we found no differences in DMT plasma concentrations between groups of normals, chronic schizophrenics, acute schizophrenics, and psychotic depressives. In fact, we were unable to detect DMT in plasma at all except in one normal and one psychotic depressive. Kaplan et al. observed that DMT blood concentrations following administration of a psychoactive DMT dose (0.7 mg/kg) to human subjects were low, peaking at less than 100 ng/ml at ten minutes post-dose (the time of the greatest subjective response) and dropping off rapidly thereafter.

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One possible explanation for the failure to find a correlation between blood concentrations of DMT and schizophrenia may be that endogenous DMT concentrations are exceedingly small. Any differences in DMT concentrations between normals and schizophrenics would be missed if the higher level were still below the detection limit of the assay employed. Several refinements in the original assay procedure improved the detection limit to 0.05 ng/ml whole blood. In this study 50% of the patients and 40% of the controls gave measurable DMT concentrations. In the work described here, the assay procedure has been modified extensively, resulting in a practical sensitivity limit for DMT of 10 pg/ml whole blood.

It has been hypothesized that phenylethylamine (PEA), a naturally-occurring amine, may be of etiologic importance in some cases of schizophrenia. PEA is structurally and pharmacologically similar to amphetamine. Furthermore, normal persons who ingest amphetamines in sufficient doses may develop a symptomatology that is clinically indistinguishable from paranoid schizophrenia.

In animals PEA produces stereotypies very similar to those produced by amphetamine. Both amphetamine- and PEA-induced stereotypies in animals are blocked by neuroleptics that are also clinically useful in treating schizophrenia. The stereotypies are blocked by doses approximately equal to the neuroleptics' relative potencies in schizophrenia.

To investigate the relationship between PEA and schizophrenia we collected 24-hour urine samples from 23 chronic schizophrenics and 23 normals. All patients received a RDC diagnosis of chronic schizophrenia and had been chronically hospitalized for at least one year without return to premorbid level of functioning. Eleven patients were subgrouped as paranoid schizophrenic and 12 as nonparanoid schizophrenic as determined by historical review, semistructured interview, and staff consensus diagnoses based on DSM II categories. While many patients were on phenothiazines, we found that phenothiazines did not consistently alter urinary PEA levels in eight patients studied on and off medication. The one-way analysis of variance test was significant for 24-hour PEA urinary excretion for paranoid, nonparanoid, and normal subjects (F=6.21, p<.0043, with Brown-Forsythe Unequal Variance correction F=3.98, p<.04). The 11 paranoid chronic schizophrenics' mean ± SEM PEA excretion of 18.1±5.4 ng/24 hrs was significantly higher than the 23 normal controls' excretion of 5.6±.5 ng (two-tailed t-test for unequal variances p<.044). The nonparanoid subgroup (8.6±2.4) was not significantly higher than the control group.

Since MAO is decreased in the platelets and lymphocytes of some chronic schizophrenics, particularly paranoid schizophrenics, it is possible that such a reduction might represent a decreased capacity to metabolize PEA and thereby lead to PEA's accumulation. The finding of increased PEA in urine of chronic schizophrenics, offers some indication that PEA may be an endogenous amphetamine.

Phenylacetic acid. A rapid, sensitive method for the quantification of free and total PAA in 10 µl of urine by mass fragmentography was developed. The method is very reproducable showing an interclass correlation of 0.97 seg at .001 on nine samples done three times. Total PAA was determined in 12 and 24-hour collections stored at -40°C. All determinations were done in duplicate (410 determinations, interclass correlation .92-.97 on duplicates).

The mean ± SEM excretion of total, conjugated and free PAA in urine of twenty-four normal adult volunteers was respectively 137.4±15.8, 128.8±15.0, 8.49±0.99 mg/24hr. Free PAA constitutes less than 10% of the total (free plus conjugated) urine excretion of PAA.

The schizophrenic patients had a significantly lower (+=2.05, $p<.05$) urinary PAA than normals (schizophrenics 95.5±15.5 mg, N=24, and normals 146.2±19.7, N=22). Eight patients consistently showed extremely low PAA excretion, and only three normals showed low excretion.

Using the matched samples test, no significant difference in PAA was found between the 12-hour day urine collections (9:00 a.m. - 9:00 p.m.) and the 12-hour night collections in normals (t=1.0). However, the day urine collections in 12 patients were significantly lower in PAA than the night collections (t=2.44, $p<.05$). No significant difference was found in urine PAA before and after ingestion of 10 ounces of chocolate (0.19 mg PEA) in five normals but headaches were precipitated in three.

To further explore our interest in phenylethylamine metabolism and psychiatric disorders, urines were collected from 9:00 a.m. to 9:00 p.m. on one day and 9:00 p.m. to 9:00 a.m. the following day from 13 normal adult volunteers working at the National Institute of Mental Health. After collection, the urines were mixed with 10 ml 10% EDTA, the volume measured and aliquots frozen at -40°C until analyzed. These urines were analyzed for total PAA. Twenty-four hour urines were also collected from 24 normal subjects and analyzed for free, total and conjugated PAA. The addition of 10% EDTA did not alter the urine pH. All urines were individually checked and found to have pH values between 5 and 7 with a mean of around 6. Storage of urine at -40°C did not appear to effect free PAA nor PAG concentrations. Analysis of some urines repeated about 6 months apart gave essentially the same results.

To test the reliability of the assay, samples from ten individuals were aliquoted into two sets of vials and blindly analyzed one week apart. The intraclass correlations for total, conjugated and free PAA were 0.98, 0.85 and 0.95 respectively. On the other hand, duplicates analysis of several urines carried out in the same batch showed correlation coefficients well over 0.95. The method described here is currently being used to evaluate PAA excretion in mental illnesses and is linear for all values so far examined. We have recently completed the analysis of urines obtained from patients on a variety of antipsychotic drugs and failed to observe a single sample with peaks that emerge near or close to those corresponding to PAA and PAA-d_7.

C. Animal

Six individually caged juvenile Macaca mulatta (Rhesus monkeys) were studied during two sessions before and four sessions after IM injections of PEA. During each session, two observers who were blind to treatment conditions, counted checking (a stereotyped form of hypervigilance manifested by abrupt changes in visual field) and 27 other discrete categories of behavior. Differences between pre-drug and post-drug behavioral counts were computed for each animal.

Single injections of PEA produced dose-related (25-75 mg/kg) significant increases in checking and lip smacking (another stereotyped behavior) as well as decreases in goal-directed behaviors such as grooming. These effects, which are often accompanied by variable increases in other stereotyped behavior such as rocking, chewing, gnashing and repetitive pacing were maximal at 15 minutes post injection, still substantial at 30 minutes, and mostly dissipated by one hour. These effects are similar to those induced by the chronic administration of amphetamines. Of interest is that unlike amphetamines, consistent tolerance did not invariably develop to the repetitive administration of PEA (75 mg/kg t.i.d.) for four or nine days.

Pretreatment with alpha-methyl-para-tyrosine (AMPT) (125 mg/kg), but not with phenobarbital (25 mg/kg) substantially reduced the characteristic effects of PEA. However, when the same AMPT pretreatment was followed by a subthreshold dose of L-DOPA (5 mg/kg) typical PEA effects were restored. This finding suggests that at least some PEA effects require intact dopaminergic mechanisms and that AMPT does not block PEA receptors.

In a present study, we demonstrated that pretreatment with either a Type A MAOI, clorgyline, or a Type B MAOI, pargyline, markedly reduced the dose of PEA required to produce checking and a variety of other behavioral changes. The present results indicate that pretreatment with MAO inhibitors, clorgyline and pargyline, potentiate the effects of PEA on Checking. That is, doses of PEA (5 or 10 mg/kg), which we previously showed to be without effect, now produced significant Checking values in pretreated animals. Neither inhibitor by itself produced Checking.

Although PEA is a better substrate for MAO Type B than for Type A, both clorgyline (a Type A inhibitor) and pargyline (a Type B inhibitor) appeared to potentiate the behavioral effects of exogenously administered PEA on Checking. The reasons for these observations are not fully known at this time. Previous studies have suggested that PEA releases dopamine: i.e., the behavioral effects of PEA were blocked not only by pretreatment with alpha-methyl-para-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase but by pretreatment with dopamine receptor blockers such as chlorpromazine, pimozide and haloperidol. Since both Type A and Type B MAO inhibitors may inhibit the metabolism of dopamine, the present results are compatable with the hypothesis that PEA's effects are mediated by dopamine. Further studies would be required to substantiate this hypothesis.

D. Convulsive Properties

Methionine has been found to alter the behavior of animals and to adversely affect the behavior of schizophrenic humans. It has been suggested that these effects are due either to (1) increased availability of methyl groups for the production of methylated amines; or (2) to the accumulation of toxic metabolites of methionine, such as homocysteine and homocysteic acid, which have convulsive properties. If this latter hypothesis is correct, methionine itself would be expected to have the ability to promote convulsions, either alone or when administered to animals that have been "primed" with subconvulsive dosages of other known convulsants.

Initially, the inability of methionine alone to induce convulsions was demonstrated by (1) administrating solutions containing 250, 500, or 1000 mg/kg of methionine (i.p. or s.q., N=243) and observing the animals for four hours; (2) administering suspensions of 1,2, or 4 mg/kg of methionine i.p. (N=8 per treatment), and observing the animals for four hours; and (3) infusing methionine (50 mg/ml) into mice via the tail vein at varying rates in total dosages of up to 10 gm/kg (N=8). In no case was a convulsion, tremor, or any convulsive-like phenomenon observed in any mouse given methionine alone. Alterations in behavioral activity were observed; the 250 mg/kg dosage caused small increases in behavioral activity, while the larger dosages (500-4000 mg/kg) caused decreases in activity. However, when methionine (1000 mg/kg) was given to animals pretreated with subconvulsive or threshold convulsive doses of homocysteine or pentylenetrazol, a pronounced enhancement of convulsive responses was observed: homocysteine seemed to be more effective than pentylenetetrazol in this respect. The convulsion-promoting effect of methionine was not shared by other amino acids, including glutamate, glycine, cysteine, tryptophan, and others. On the other hand, ethionine, an analogue of methionine, was found to enhance convulsions about as effectively as methionine. These results show that methionine has convulsion-promoting properties under certain conditions, and support the suggest that the organicity produced by methionine clinically is due to the accumulation of metabolites such as homocysteine.

Anticonvulsant Properties of Betaine. Betaine has been found to block the induction of convulsions produced by homocysteine; the reason for this effect has been presumed to be that betaine acts as a source of methyl groups for the methylation of homocysteine. This hypothesis would not predict that betaine would have general anticonvulsant properties. A series of experiments was performed to test this possibility. It was found that betaine (380-1540 mg/kg) partially blocked not only those convulsions induced by homocysteine, but also the convulsions induced by electroconvulsive shock and pentylenetetrazol. If the reason for the anticonvulsant activity of betaine is indeed that it reduces the ratio of free homocysteine to free methionine in the body, the present finding suggest that homocysteine plays a role in the regulation of neuronal excitability under "normal" circumstances; e.g., in the absence of an administration of exogenous homocysteine.

XVI. Low Platelet Monoamine Oxidase Activity and Schizophrenia

The finding that blood platelet monoamine oxidase (MAO) activity is low in some schizophrenic patients has led us and others to inquire as to its significance. As part of this endeavor, there has been a continuous effort to determine if this low enzyme activity was due to one of the large number of artifacts that have plagued the investigation of biological factors related to psychiatric disorders. The enzyme has been assayed in the laboratory of Dr. Dennis Murphy, who, in addition to his interest in the enzyme in schizophrenia, has been using the platelet as a model for synaptosomes.

A. MAO Assay in Blood Platelets

In our initial studies, venous blood was collected in heparin, or more recently, in Becton-Dickinson Company Vacutainer tubes containing ACD (acid-citrate-dextrose), NIH Formula A, and platelets separated and stored according to previously described methods. MAO activity was determined using tryptamine-2-^{14}C HCl (8x10-^{5}M, 8.9 mCi/mM) as substrate and was expressed as nM of tryptamine coverted per mg of platelet protein. The product was indoleacetaldehyde, whose formation was linear with time and enzyme concentration.

During the last several years, our samples were also obtained in the ACD solution, but the MAO activity was determined in platelet rich plasma. The present assays uses benzylamine-^{14}C (4x10-^{4} mCi/mM) as substrate, and is expressed as nM of product per 10-^{8} platelets per hour. Benzylamine is a good substrate for both platelet MAO and an entirely different enzyme occurring in the plasma. Because of this, platelet rich plasma samples with and without the MAO inhibitor pargyline are run. Pargyline (2.4x10-^{4}M) completely inhibits the platelet enzyme without affecting the plasma enzyme. The value of sample with the inhibitor is substracted from the sample without--the difference being the platelet activity.

The advantage of the platelet rich plasma assay over the previous one is in not having to determine protein which can easily be contaminated with red and white cells.

B. Stability of Enzyme

Because at times it is not possible to process blood samples or run enzyme assays immediately, it is necessary to know the stability of the enzyme. Comparisons of blood samples processed immediately or kept on ice or at room temperature for five hours revealed no change in activity. Similarly, once the platelet rich plasma is separated, MAO activity remains stable for at least 30 days when kept at -70°C.

C. Comparison of Assays

Pearson correlations were run for 48 normals and 20 chronic schizophrenics between the two assays. The correlation for the normals was .85

while that for the chronic schizophrenic patients was .76. For the combined group the correlation was .83. All our platelet MAO activities are expressed in benzylamine values with a correction (derived from the normals) for values determined using the tryptamine assay.

D. Subjects

Normal bloods were drawn from normal volunteers and Hospital personnel at both the Clinical Center in Bethesda, Maryland and the William A. White Building of Saint Elizabeths Hospital. The bloods from the acute schizophrenics largely came from a research ward at the Clinical Center while those from the chronic schizophrenic patients came from special research wards at the William A. White Building.

E. Age and Sex

Others have reported that females had 10 percent higher platelet MAO activity than males. Our sample females have a slight but consistently higher MAO activity although this difference is statistically significant only for the young adult group.

F. Drugs

Although early experiments with chlorpromazine in intact systems indicated that chlorpromazine had some effects on amine degradation, this seemed to be due to poor amine accessibility to the enzyme because of alterations in the cell membrane. Edwards and Burns (1974) found that the I50 for amitriptyline was 2×10^{-4}M for human platelet MAO *in vitro* when benzylamine was used as a substrate. The inhibition was competitive.

In studies on patients given neuroleptics, there does not appear to be a consistent difference between when they are on neuroleptics or off, or between patients on neuroleptics and their siblings not receiving drugs.

G. Hormones

One study using only two points found no alteration in platelet MAO activity during the human menstrual cycle. In another study which utilized samples collected three times a week, there were small (23 percent) peak to trough variations in activity in the majority of women. The peak MAO activity occurred during the preovulatory interval and the nadir occurred five to 11 days later. A small number of patients taking prednisone have been seen and have normal MAO activities. Similarly, there does not appear to be any correspondence between plasma thyroxin and triiodothyronine concentrations in psychiatric patients with low and normal MAO activities.

H. Other Factors

The hematocrit, hemoglobin and platelet counts, serum iron, and iron binding capacity were not systematically different in patients with low and normal MAO activity. Other platelet enzyme activities including succinate dehydrogenase, lactate dehydrogenase and cytochrome C reductase were no different in chronic schizophrenic patients with low MAO than they were in controls.

I. Chronic Schizophrenics

To date we have studied platelet MAO activity in 104 male chronic schizophrenic patients. Their mean ±S.E. MAO activity was 7.86±.56 while the value for 348 normal males (ages 18-50) was 11.04±.29. This difference using a two-tailed "t" test, was significant at $p<.001$. The mean for the 15 female chronic schizophrenics was 7.04±1.06 while that for 332 normal females was 13.29±0.29 ($p<.001$).

The effect of chronic hospitalization on platelet MAO was studied at the Palo Alto VA Hospital where 50 normal controls were compared to 16 patients hospitalized for traumatic accidents to their spines. There was no difference between MAO activity between groups but 39 chronic schizophrenics had decreased platelet MAO activity.

At least 26 studies have been published concerning the enzyme, monoamine oxidase (MAO), in platelets and white cells of chronic schizophrenic patients. Although at times the deviation from controls has been very small, in all but 4 instances a decrease in MAO activity has been found. In 19 studies, the reported decrease was statistically significant. Using the sign test, the chance of 22 of 26 studies demonstrating a decrease is less than 1 in 4,000 and the possibility of 19 of 26 being statistically significant at the 0.05 level by chance alone is less than 1 in 10 (binomial test). In spite of this evidence, the finding continues to be thought of as controversial. We believe that what is controversial about the finding is not whether or not platelet MAO activity is decreased in some schizophrenics, but 1) to what degree, 2) in which patients, and 3) what, if any, biological significance this decrease might have.

J. Acute vs. Chronic Schizophrenics

To determine whether acute schizophrenics have a reduction in platelet MAO as did the chronic schizophrenics, platelet MAO of 27 acute schizophrenics were studied. The 10 male acute schizophrenics (ages 16-30) had a mean platelet MAO activity of 10.68±78 which was no different than that of normal males. The 17 female acute patients had a mean of 13.85±1.12 which was no different from that for the normal females.

K. Subgroups of Chronic Schizophrenics

Platelet MAO in two groups of chronic schizophrenic patients ($N=35$, $x=7.52$; $N=42$, $x=7.86$) was significantly lower than in controls ($N=680$, $x=12.14$). Values for those patients diagnosed as paranoid, ($N=8$, $x=4.47$) were significantly lower when compared to non-paranoid schizophrenics ($N=27$, $x=8.43$) in a retrospective study. These findings were confirmed in a separate prospective study of 42 chronic schizophrenics (paranoid and paranoid features N=24, x=6.40 compared to non-paranoid N=18, x=9.81). Schildkraut found low platelet monoamine oxidase to be associated with auditory hallucinations and delusions, usually of a persecutory quality. Twelve out of 16 of these low monoamine oxidase patients were designated paranoid. Reduced platelet MAO activity in normal subjects appears to be associated with a greater tendency towards psychopathology. These data could indicate that paranoid schizophrenia may represent a separate disorder from the other schizophrenias. The correlation between paranoid schizophrenia and low platelet MAO activity suggests some biochemical difference between the different subgroups of schizophrenia.

L. Specificity with Regard to Other Illness

Since the various psychiatric diagnostic classifications are thought to represent different entities, a defect in one should not be seen in the other. The affective illnesses are the group we most diligently contrasted with the schizophrenias.

Sixteen male and 13 female unipolar depressed patients were found to have platelet MAO activity that was no different from our controls while 16 male and 33 female bipolar I depressed patients had reduced activity ($p < .001$). The bipolar patients had a negative skew which was not as large as that of the chronic schizophrenics. Using six units of MAO for the male normals and eight units for the normal females as a point of cut-off, a point under which less than 10 percent of normals fit, 39 percent of male chronic schizophrenics and 60 percent of the female chronic schizophrenics are found (chi square $p < .001$). The bipolar patients also differed from normals ($p < .001$ males; $p < .02$ females). The male bipolar patients were not significantly different from the male chronic schizophrenic patients, but the percentage of female chronic schizophrenics with MAO activity below eight units was greater than the female bipolar I patients. Thus, although bipolar I patients using the arbitrary cut-off have a mean MAO which is low, it is not clear whether they represent a separate group from the chronic schizophrenic patients.

M. Twins Discordant for Schizophrenia

To exclude the possibility that the low platelet MAO activity seen in the chronic schizophrenics was caused by nongenetic factors as opposed to genetic ones, we studied monozygotic twins discordant for schizophrenia. If the low platelet MAO activity is due to some aspect of being schizophrenic, low platelet MAO should only be present in the schizophrenic twin. Thirteen schizophrenic index twins, all of whom had been hospitalized at least once for schizophrenia and had been extensively studied by Dr. William Pollin and his associates, were examined along with their non schizophrenic co-twins. At the time of the study, one patient resided within a hospital while five were in remission. Four of the patients had had acute forms of schizophrenia and six patients were not receiving any antipsychotic medication. The nonschizophrenic co-twins had never been hospitalized for a behavioral disorder and were functioning well within their families and communities, except for one individual with borderline psychosocial adjustment.

Only two twin pairs were living in the same household and nine of the co-twins were living in different cities. It was, therefore, necessary to obtain and prepare the blood samples at various facilities throughout the country. Because of this, normal control samples were obtained at the same time as those from the twins. They were coded and shipped to the Laboratory. All samples were batched together before assay. (In all studies samples are run in a manner so that their origins are unknown to all investigators until the final calculations are made.)

The MAO activity of the 23 normal controls was the same as that obtained at that time for previous controls (16.02). The schizophrenic twins (9.88, $p<.005$) and nonschizophrenic twins (12.08, $p<.05$) were significantly lower than the normals, but there was no difference between the twin groups. There was a significant Pearson correlation ($r=.67$, $p<.01$) between the MAO activities in the schizophrenic and nonschizophrenic twins.

There were four pairs of twins whose MAO activities were especially low. The indexes of all four of these twin pairs were chronic schizophrenics while the more acute patients had MAO activities closer to normal. The severity of impairment (based upon number and duration of hospitalizations) was rated on a 5-point scale for the schizophrenic twins by an investigator with no knowledge of the platelet assay. A forced rank order was then made between the numerical ratings, with the highest number given to the most ill patients. Using this order, and comparing it to the MAO activities, a Spearman rank order correlation of -.54 ($p<.05$) was found.

Platelet MAO activity was also examined in nine monoxygotic and 10 dizygotic normal twins. The intraclass correlation coefficient was 0.88 for the monozygotic and 0.45 for the dizygotic twins. The correlations were significantly different from one another (Mann Whitney U $p<.001$). Same-sex siblings matched for age and sex were about the same as the dizygotic twins while unrelated similar to those reported by Nies, Robinson, Lamborn and Lampert (1973) for normal monozygotic and dizygotic twins using benzylamine instead of tryptamine as a substrate.

Taken together this data indicates that platelet MAO activity is in a large part determined by genetic factors and that the low platelet activity seen in the chronic schizophrenics is not secondary to being ill, but is genetically related to the liability to be schizophrenic.

N. Family Studies

In order to further study the relationship of low platelet MAO to schizophrenia the blood of first-degree nonschizophrenic relatives of index schizophrenics (N=16) was collected. Dividing schizophrenic indexes at their median, the low indexes had a mean of 4.64 and the high indexes had a mean of 10.81. The mean MAO activity for the low index first-degree relative was 8.56. The mean activity for high index first-degree relatives was 11.84. The difference between the means for the low vs. high first-degree relatives was significant ($p < .02$; Mann Whitney U). No attempt was made in this small sample to account for sex differences. These differences between nonschizophrenic first-degree relatives of high and low schizophrenics is another indication of the genetic nature of platelet MAO activity.

Ongoing family studies for the past two years presently include studying MAO in platelets and lymphocytes in first-degree relatives of schizophrenics. At present, findings are inconclusive. As well, a collaborative effort is being established with other Divisions of the Hospital to study the inheritance of schizophrenia by hospitalizing first-degree relatives with schizophrenia.

O. Physical-Chemical Studies

In one study, we determined the rate of heat inactivation of the MAO in a group of chronic schizophrenic patients (N=22) and normal subjects (N=13). Aliquots of platelet MAO were subjected to different temperatures between 56 and 62 C for five minutes. Analysis of the data revealed no significant differences in the rate of heat inactivation between the normal and schizophrenic groups confirming an earlier study from this laboratory. However, when the schizophrenic group was divided into paranoid schizophrenic and chronic undifferentiated schizophrenic (CUS) groups, differences became apparent. The MAO in the paranoid group was most resistant to heat while the MAO in the CUS group was the least resistant; the MAO in the normal group was intermediate in its rate of heat inactivation. There was a significant difference ($p<.05$) between the paranoid and nonparanoid (CUS) groups. Neither schizophrenic group differed significantly from the normal group. However, the combination of the normal plus the CUS group differ significantly from the paranoid group ($p<.035$).

In a second study, we have attempted to replicate a finding reported by Berrettini and Vogel that an inhibitor of platelet MAO is present in the plasma of schizophrenic patients that have low MAO activity. If their report were correct, it would provide a mechanism that would explain the reduced MAO activity found in schizophrenic patients. However, we have not been able to replicate their results. In fact, in five of six experiments we have found a slight increase in MAO activity when normal platelet MAO is preincubated with plasma from schizophrenic patients (effect of chronic plasma on Km and Vmax, 1.5±0.05 and 22.1±2.46; effect of schizophrenic plasma on Km and Vmax, 1.8±0.28 and 24.9±1.57). We would conclude from our studies that an inhibitor does not appear to be present in schizophrenic plasma.

In a third study, we are attempting to replicate the finding of a reduced Km in the platelet MAO in a group of paranoid patients compared to a group of normal subjects. Although our preliminary findings are in the right direction, certain methodological problems have become apparent and need to be resolved before we can complete the study.

If this finding can be firmly established in the schizophrenic group, we then plan to proceed and to measure the Km in a group of alcoholic patients and in a group of bipolar depressive patients. The overall purpose would be to determine if the decrease of Km in the schizophrenic patients observed is unique to that patient group or is shared by the other two patient groups that have also been reported to have a reduced platelet MAO activity.

Heat Inactivation: Most studies have reported that chronic schizophrenic patients have reduced platelet monoamine oxidase (MAO) activity. Possible environmental and genetic factors that might cause the lower activity have been the subject of intense investigation. On the one hand, most environmental factors, such as drugs and hospitalization that might cause the decreased MAO activity have been excluded. On the other hand, it appears that the level of MAO activity in both normal and schizophrenics is, at least in part, due to genetic factors. Given the possibility of a genetic contribution, it seemed reasonable to determine if the MAO itself was altered. To determine this, we have examined some physical-chemical properties of MAO. We and others have shown that with some substrates the Km is altered in the MAO of chronic schizophrenic patients. In the present study, we have attempted to examined the rate of heat inactivation of platelet MAO in a group of normal subjects and chronic schizophrenic patients.

Berrettini and Vogel describe evidence for an inhibitor of platelet monoamine oxidase (MAO) in the blood of chronic schizophrenic patients. They suggest that this endogenous inhibitor may be responsible for the decreased platelet MAO activity reported in chronic schizophrenics. We, however, failed to demonstrate the presence of this inhibitor.

The blood from eight patients with low MAO activity was used. The plasma from two patients was provided by Dr. Berrettini. The other six patients were hospitalized at Saint Elizabeths Hospital where they received a diagnosis of schizophrenia both by Feighner and Research Diagnostic Criteria. The patients who satisfied these criteria had a chronic illness and were unable to function in the community. Additionally, the clinical staff who best knew the six patients rendered a consensus subtype diagnosis of paranoid schizophrenia based on DSM-II categories. Since Berrettini and Vogel did not use drug free patients and since neuroleptic drugs seem to have little effect on MAO activity, the patients used in the present study were not neuroleptic free. Staff and laboratory personnel were used for the control population. The procedure for determining the presence of an inhibitor was that employed by Berrettini and Vogel. They indicate that the inhibitor is present in the platelet-poor plasma (PPP) of schizophrenic patients who have low platelet

MAO activity. To briefly describe their procedure, three aliquots of platelet-rich plasma (PRP) from a normal were used in each experiment. One aliquot of the normal PRP was unmixed. The second PRP aliquot was mixed with PPP that was obtained from a second normal control which presumably contained no inhibitor. The third PRP aliquot was mixed with the schizophrenic PPP. All three samples were incubated for 10 min. at 37 . The samples were centrifuged to obtain the platelet pellet which was then washed and resuspended in buffer. The kinetic parameters (Km and Vmax) for the three samples were determined.

The enzymatic assay method of Wurtman and Axelrod was used. Radioactive tyramine (tyramine [$1-^{14}C$]) and radioactive dopamine (dihydroxyphenylethylamine hydrobromide, 3,4 (ethyl-^{14}C)) were obtained from New England Nuclear. When tyramine was employed as a substrate, five concentrations were used that range from $10X10^{-4}$ to $1X10^{-4}$M. The reaction was stopped with perchloric acid and the product was extracted with toluene. When dopamine was employed as a substrate, five concentrations were used that ranged from $8X10^{-4}$ to $0.5X10^{-4}$ M. The reaction was stopped with hydrochloric acid and the product extracted with toluene: tetra-hydrofuran (2:1, V:V). Protein was determined by the method of Lowry et al.

The Km's and Vmax's were obtained by fitting data with a PDP-11 computer to a rectangular hyperbola according to the iterative procedures of Bliss and James. This method yields the same results as those procedures developed by Cleland. The data were then further analyzed with a two-tailed paired t-test.

The data indicate that there were no significant differences among any of the groups in either the Km or Vmax measurements. Berrettini and Vogel reported that treatment of normal platelets with schizophrenic platelet-poor plasma reduced both the Vmax and Km of normal MAO. We did not find reductions; if anything, our data indicate that the schizophrenic plasma produced nonsignificant increases in MAO activity. More specifically, the schizophrenic plasma produced increases in the Vmax in 6 out of 8 cases when tyramine was used as a substrate ($p < .06$). In addition, the two schizophrenic plasmas provided by Dr. Berrettini also produced increases in the Vmax.

It is not clear why we were unable to replicate the findings of Berrettini and Vogel. If there is an inhibitor, there must be factors affecting the procedure of which Berrettini and Vogel are not aware. One possible factor involved could be the procedure used in analyzing the data. We used the objective mathematical method of Bliss and James. Berrettini and Vogel used Linewearver-Burk plots in which the straight line was fitted by eye to obtain their Vmax and Km values. This plotting procedure has been shown to be subject to substantial error. Dowd and Riggs conclude that, "The marked inferiority of the Linewearver-Burk plot strongly suggests that it should be abandoned as a method for estimating Km and Vmax from unweighted points, whether the points are fitted by eye or by the method of least squares." Furthermore, if Berrettini and Vogel did not plot their data as a blind procedure, an additional error could have been introduced.

P. Relationship of Platelet MAO in Brain MAO

The number of types of MAO present in the brain is an unresolved question. Original studies indicated that there might be as many as four isoenzymes of MAO within the brain; however, Houslay and Tipton (1973) suggested that the multiple enzymes found using polyacrylamide-gel electrophoresis might be produced artifactually in a preparatory step. The authors state that these multiple MAO forms may represent a single protein with varying amounts of phospholipid attached; the amount of phospholipid may, in turn, determine the enzyme mobility during electrophoresis.

Another approach to this problem has come from the finding that selective inhibition of MAO can be used to identify multiple forms of MAO. Clorgyline was the first MAO inhibitor employed in this manner. It was found that tissue homogenates pretreated with increasing concentrations of clorgyline produced stepwise inhibition to the substrate tyramine, suggesting the presence of an enzyme sensitive to the inhibitor and one relatively resistent to it. Considerably more work has been done to identify these enzymes and for convenience they are called Type A and Type B.

The preferred substrates for the Type A MAO are norepinephrine, serotonin, and normetanephrine, while for the Type B they are benzylamine and -phenylethylamine. Dopamine, tyramine and tryptamine are common substrates. Clorgyline, Lilly 51641, and harmaline are specific inhibitors of Type A while deprenyl and pargyline inhibit Type B. Most of the other MAO inhibitors used in clinical medicine, however, are nonspecific. Platelet MAO, forming one band on electrophoresis, appears to be a single enzyme with Type B characteristics. That is, the platelet enzyme demonstrates only a single sigmoid curve for inhibition using deprenyl, pargyline, or clorgyline. Furthermore, the platelet enzyme is 200-fold more sensitive to inhibition by deprenyl than clorgyline. The conclusion that the platelet enzyme is a simple Type B MAO is also supported by the high activity of the platelet enzyme with the relatively specific MAO-B substrates, benzylamine and β -phenylethylamine, in contrast to its lesser activity with serotonin, an A substrate.

Q. Function

To determine whether or not the low platelet MAO activity might have a functional significance, platelet-rich plasma was obtained from 20 normal controls (age 29.6±1.1) and 16 chronic schizophrenic patients (27±1.4). Since plasma serotonin is almost entirely contained in the platelet the values were expressed in platelet units (ng/108 platelets). The platelet counts for the normals and schizophrenics were the same. When the patients had been off phenothiazines (N=12) for 30 days or longer the serotonin concentration was higher (80±4), while when the patients were taking phenothiazines (N=12) their platelet serotonin concentration was normal (90±9). The reason for the discrepancy between patients taking and not taking phenothiazines may be due to the latter's ability to block serotonin uptake into platelets. Nevertheless, since serotonin is not a good substrate for platelet MAO (Km 10^{-3}) other substrates might be much more affected.

Increases in plasma prolactin concentrations produced by alpha-methyl-para-tyrosine, a catecholamine synthesis inhibitor, varied inversely with baseline platelet monoamine oxidase activity in 12 chronic schizophrenic patients. Two subsequent studies with 39 normal volunteers and 19 unmedicated chronic schizophrenic patients were conducted to test for a relationship between platelet monoamine oxidase activity and plasma prolactin concentration. Plasma prolactin concentrations were found to vary directly with platelet monoamine oxidase activity in normals with low monoamine oxidase activity and patients with chronic schizophrenia. No relationship was found in normals with high platelet monoamine oxidase activity. These data suggest that platelet monoamine oxidase activity reflects monoaminergic activity in the tuberoinfundibular system which in turn affects plasma prolactin concentrations. This relationship may be particularly important in patients with low platelet monoamine oxidase activity such as some chronic schizophrenics.

R. Subclass of Schizophrenia

In 1974-1975 we undertook a restrospective study of 43 patients we had examined to look at who the patients were with low platelet MAO. Information was obtained regarding circumstances and age at onset of illness, presenting problems, diagnoses and family history of mental illness. The patient's medical and surgical history was reviewed, as well as laboratory data which included EEG's and skull x-rays. Mental status examination on admission and throughout hospitalization was summarized, as well as changes observed on medication. This information was gathered and coded without knowledge of platelet MAO activity.

Two types of statistical analyses were employed. The first approach correlated MAO activity with a variety of clinical variables using the Pearson product-moment correlation coefficient. The second approach looked for differences between groups of patients with low and high MAO activity. In the second approach, the MAO value of 9.16 units was arbitrarily chosen to divide the chronic schizophrenic patients into high and low MAO groups (12 patients in the high group; 31 patients in the low group). 9.16 is two standard errors above the female chronic schizophrenic mean (8.04 and slightly higher than two standard errors above the male schizophrenic mean (7.86). The term "high MAO" in this study refers to high MAO activity for chronic schizophrenic patients. The high MAO group has a mean ± SEM of 13.54±1.06, which is comparable to values for the normal population. The low MAO group had a mean of 5.47±.37. All patients were diagnosed chronic schizophrenics with a mean hospital stay of 9.3 years, although length of hospitalization within the sample did not correlate with MAO activity (r=.11). MAO activity did not correlate with age at onset of symptoms (r=.06), age at first hospitalization (r=.12), length of hospitalization (r=.11), or with problems at work (r=.20), school (r=.07), or marriage (r=.12).

Schizophrenic patients with low MAO had a history of more seizures ($r=.31$, $p<.05$) than patients with high MAO. Of the 41 patients from whom we had an adequate history of seizures, 8 of 30 low MAO schizophrenics had at least one seizure during hospitalization, compared to 1 of 11 high MAO schizophrenics. The high-low difference was not significant using the Fisher Exact Probability Test. None of the patients was on anticonvulsive medication. The possibility of abnormal brain dysfunction correlating with low MAO was reflected in the significant negative correlation between MAO and the number of abnormal EEG's ($r=.32$, $p<.05$). Of those 36 patients who had EEG records available, 10 of the 25 low MAO schizophrenics had at least one abnormal EEG, compared to none of the 11 high MAO schizophrenics (Fisher Exact Probability Test, $p<.01$). The data indicate that low MAO activity in chronic schizophrenics is associated with an increase in abnormal EEG's and perhaps an increase in seizures. This abnormal brain activity is, however, not associated with other medical or asurgical problems, or with any abnormalities in routine blood studies.

In regard to the mental status examination, the patients with low MAO activity were reported to have more ambivalence ($4=.32$, $p<.05$) but not any difference in severity or frequency of thought disorder ($r=.02$), associational defects ($r=.10$), autistic behavior ($r=.03$), hallucinations ($r=.09$), instances of self-mutilation ($r=.04$) or suicide attempts ($r=.24$). No evidence for differences in treatment response related to MAO activity was found ($r=.05$). Mental illness in relatives did not correlate ($r=.19$) with platelet MAO activity and was not different between the high and low MAO groups. However, it might be impossible to determine such a correlation from the relatively small sample. This is compounded by the inherent difficulties in determining a complete accounting of family incidence of mental illness from chart analysis.

All of the paranoid schizophrenics had low MAO. None of the high MAO activity schizophrenics received a DMS II diagnosis of paranoid schizophrenia during their hospital stay; 7 of the 30 low MAO group did (Fisher Exact Probability Test $p<.05$.) This difference was further substantiated when the means of MAO activity were compared between those who received a diagnosis of chronic paranoid schizophrenia and those who did not. The paranoid schizophrenics have a mean MAO activity of $4.8\pm.46$, which is significantly lower than the nonparanoid schizophrenic values of $8.6\pm.75$ (t-test, $p<.03$). This difference was also reflected in symptoms characteristic of chronic paranoid schizophrenia, such as paranoid delusions and increased hostility, but not in other less specific symptoms.

From this study emerged a picture of a low platelet MAO activity schizophrenic who is more frequently diagnosed as paranoid, as having more paranoid type delusions, and as having abnormal brain activity reflected by increased incidence of seizures and abnormal EEG's.

In order to further examine these findings, 42 new patients were selected on the basis of definite diagnosis of schizophrenia who had active symptoms but without regard to subtype. Patients with a diagnosis of organic brain syndrome or mental retardation or other cerebral pathology, including severe alcoholism, were excluded in order to focus on the paranoid dimensions. The 42 subjects were drawn from a sample of 65. Fifteen were excluded because of their history, eight others met criteria but refused to take part in the study. Seven of these eight refused because of apparent suspiciousness or distrust of the experimental procedure.

All patients met Research Diagnostic Criteria and 41 of 42 met Feighner's criteria for a diagnosis of schizophrenia. The mean age was 35, mean length of hospitalization was 401 months, and all patients had, at one time, received phenothiazine treatment. All patients had a chronic course and 40 of the 42 had a gradual or insidious onset of psychosis. Twenty-nine were males and 13 females. MAO values were 6.7 and 10.6 units for males and females, respectively, compared to mean MAO's of 11.04 and 13.29 for normal males and females. The difference between the schizophrenics and normals was significant at $p<.01$. The clinical staff who best knew the patient rendered a consensus diagnosis based on DSM II categories. A secondary diagnosis was reached for cases of chronic, undifferentiated schizophrenia. All patients underwent a semi-structured interview by Rosenthal. Interviews were conducted at a time as close as possible to blood drawings, and for most patients were done on one occasion over a 60-minute period. Repeated interviews were occasionally required to obtain the necessary information.

The information was gathered and coded without knowledge of MAO values. The data indicate that patients diagnosed as paranoid schizophrenics had a mean platelet activity MAO level of 5.4 for males and 8.9 for females, compared with 8.6 and 12.1 for nonparanoid male and female schizophrenics. Patients with secondary paranoid features had MAO values of 5.6 for males and 9.1 for females. Values for both paranoid patients and patients with secondary paranoid features were significantly different from those of nonparanoid patients ($p<.01$).

The variables which best diagnostically divided the paranoid from nonparanoid patients by a linear discriminate function were: degree of suspiciousness, preoccupation with and systematization of delusions, delusions of control, stereotyped behavior, intact memory, rigidity of ideas, and communication characterized by evasiveness and misleading responses. These diagnostically discriminating items are similar to items on the Venables Scale for Paranoid Schizophrenics except for grandiosity which was not a good discriminator in our study.

These prospective findings confirm the retrospective chart analyses. Chronic schizophrenics had lower platelet MAO activity than normals. Paranoid schizophrenics had an even lower MAO activity level, whether the paranoia was determined by clinical diagnosis, by interview, or by a paranoia dimension scale.

S. Discussion

What does it mean to have low platelet MAO activity in view of seemingly normal brain MAO activity? The argument that post-mortem measurement of brain MAO activity does not reflect its true activity because of autolysis has little support since brain MAO seems to be a very stable enzyme. It, of course, remains a possibility that we have either not measured brain MAO activity in the right region or with correct methods, an argument that is not easily dealt with.

Enzyme activity must now be measured in other regions of the body, such as the muscle, lung, liver, and kidney. If these areas turn out to have normal MAO activity while the platelet is low, intriguing explanations will be needed.

Part of the attractiveness of the concept of low MAO activity is that it is congruent with the two most generative biological hypotheses of schizophrenia. The first of these suggests that there is a functional excess of dopamine. A defect of MAO, an enzyme involved in dopamine metabolism, might be able to produce "dopamine excess." The second hypothesis suggests the formation of an hallucinogenic methylated indoleamine (transmethylation). Since the indoleamines as well as their methylated congeners are metabolized by MAO, this theory is also consistent with a finding of low MAO activity. One of many questions that needs to be answered before low MAO activity can be considered causative, is "Why don't the vast majority of people given drugs which inhibit MAO become psychotic?"

XVII. Naloxone

Naloxone was given to 14 schizophrenic patients and five patients with affective disorders were given naloxone (0.4 to 10 milligrams) or placebo intravenously in a double-blind fashion. Physicians' ratings of hallucinations, mannerisms and posturing, conceptual disorganization, psychosis, and mood did not change significantly. A single item, unusual thought content, improved significantly on the naloxone day compared to the placebo day. There was no improvement in mood in affectively ill patients rated either by themselves or by physicians. Naloxone did not markedly improve any patient studied, which suggests that the acute blockade of opiate receptors is not associated with global improvement in psychotic symptomatology.

XVIII. CAT Scan

This study involves quantitative evaluations of computerized tomographic brain scans and quantitative assessments of ventricular volume and cortical sulci size determined from their scans in chronic schizophrenic patients under age 40. This provides a non-invasive, yet accurate means for considering possible abnormalities of these parameters described in prior pneumoencephalographic studies. Preliminary findings suggest that when compared to normal controls, psychiatric patients who do not meet research diagnostic criteria for chronic schizophrenia, as a group have larger ventricles and more cortical atrophy. It is also possible that in a small subgroup ventricular enlargement is dramatic.

To investigate the possibility that lateral cerebral ventricular enlargement is associated with chronic schizophrenia, computed tomography head scans from 73 chronic psychiatric patients under age 50 were compared with 56 asymptomatic normal volunteers of similar age. Ventricular size, determined by planimetry, was significantly greater in the subgroup of 58 patients diagnosed with chronic, unremitting schizophrenia than in the control group ($p<.0001$). Forty percent of the chronic schizophrenic patients were outside the control range; 53 percent exceeded two standard deviations of the control mean. Duration of illness and length of hospitalization, variables considered to be proportional to the amount of treatment received, did not correlate with ventricular size. Although patients with a history of ECT treatment had larger ventricles than controls ($p<0.0001$). A group of seven schizophrenic patients who were not chronic also had enlarged ventricles ($p<.006$); 8 patients who were either schizo-affective or not schizophrenic did not differ from controls.

This study demonstrates that lateral cerebral ventricular enlargement is associated with chronic schizophrenia; it suggests that this finding is not a result of treatment. Computed tomography was further employed to investigate whether mild cerebral atrophy involves the cerebral cortex. The widths of the sylvian fissure, the interhemispheric fissure, and three cortical sulci were measured blindly on computed tomography (CT) scans from 75 chronic psychiatric patients and 62 asymptomatic volunteers all under age 50. Using dimensions from the control subjects to define the range of normal size for these structures, 19 (32%) of the 60 patients diagnosed with chronic schizophrenia had at least one abnormality. All 15 patients with other diagnoses were within the control range. Comparing those chronic schizophrenic patients with abnormalities to those without them, there were no significant differences in age, length of illness or treatment, and length of hospitalization. Combining the previously reported data on ventricular size with the present findings, two-thirds of the chronic schizophrenic patients had some abnormality of cerebral structure evident on CT scanning. Ventricular enlargement, however, did not correlate significantly with cortical abnormalities. It is suggested, therefore, that more than one etiology may account for the structural abnormalities found in chronic schizophrenic patients. Furthermore, we also found, totally unexpectedly, a subgroup of 10 chronic schizophrenic patients with possible pathology of the cerebellum.

The scans of five chronic schizophrenic patients were interpreted by a neuroradiologist as showing definite atrophy of the vermis; in one of these patients, the cerebellar hemispheres were also considered atrophied. Five additional scans, which were interpreted as "questionable", met objective criteria proposed for the diagnosis of vermian atrophy on a CT scan . One of these patients had a history of alcohol abuse. Since cerebellar atrophy is common in chronic alcoholics this patient was excluded from further study.

None of the remaining nine patients (four females, five males; mean age 35, range 26-44) had an identifiable cause for these findings; no patient was taking medication other than neuroleptics. Furthermore, careful neurological examination with emphasis on cerebellar function revealed abnormalities in only three patients, two with mild dysdiadokokinesis of the non-dominant hand, and one with a slight positional tremor of both hands.

A growing body of basic science research indicates that the cerebellar vermis is linked anatomically and functionally to areas of the limbic system that have been implicated in schizophrenia. Furthermore, increased dopamine in the limbic forebrain, a neurochemical abnormality consistent with the "dopamine hypothesis" of schizophrenia, has been produced in the rat by a lesion of the vermis. It has also been reported that in several schizophrenic patients, electrical stimulation of the vermis had therapeutic effects. Such observations suggest that pathology of the cerebellar vermis may be related to the pathogenesis of some schizophrenic symptomatology.

In a morphometric study of the anterior cerebellar vermis of 46 brains in the Yakovlev Collection, the area of the vermis of five of 12 brains of schizophrenic patients was smaller than any of 11 controls without psychiatric or neurologic disease and eight of nine control with other psychiatric diagnoses ($p<.02$). This finding confirms computerized tomography scan observations in live patients and indicates that some schizophrenics have structural abnormalities of the cerebellar vermis.

Four of 17 chronic schizophrenic or chronic schizoaffective patients between the ages of 20 and 35, in partial remission, and living in the community were found to have mildly or moderately prominent sulci, as disclosed by computerized tomography (CT scan). These four patients were matched for sex and age with four chronic schizophrenic or schizoaffective patients without sulcal prominence. The Halstead-Reitan battery (HRB) of neuropsychological tests, including the Wechsler Adult Intelligence Scale (WAIS), was administered to these eight patient with the examiner blind to results of the CT scan. Each of the four patients with sulcal prominence considerably exceeded an established cutoff score for brain impairment on the HRB, whereas only one of the control schizophrenics performed in the impaired range, and that just beyond the cutoff. The WAIS Verbal IQ, measured concurrently, was in the normal range for all subjects and similar for the two groups. These findings suggest that mild or moderate sulcal prominence on CT scans among young chronic schizophrenics is associated with impaired neuropsychological functioning.

XIX. Calcitonin and Feeding

Plasma calcitonin (CT) concentrations increase following a meal and following the administration of the gastrointestinal hormones of glucagon, gastrin, pancreozymin, and cholecystokinin. A series of experiments were performed to test the hypothesis that this food-induced increase in CT secretion plays a role in reducing subsequent food intake. A single S.C. injection of synthetic salmon calcitonin was found to inhibit the 24-hr intake of rats (up to 58:; $p<0.005$) in a dose-related (12.5-50 U/kg) fashion. This was accompanied by a pronounced diuresis and increase in drinking which were, however, not observed in food-deprived animals. In rhesus monkeys, a single injection of CT (30 U/kg) greatly suppressed feeding for three days and drinking for one day (both $p<0.01$). A retrospective study of humans demonstrated a 2% reduction in body weight ($p<0.03$) during the 12-36-hr following a single S.C. injection of CT (2 U/kg).

To test the hypothesis that inhibition of feeding was due to a direct action on the CNS, we administered calcitonin to rats by intraventricular injection in dosages approximately 25 - 50 fold smaller than those that inhibited feeding when administered peripherally. Intake of food and water, excretion of feces and urine, and body weight were recorded during the two days prior to and after the injections.

The findings reported lead us to speculate that calcitonin has a specific physiologically relevant action in the CNS. We suggest this because: (i) inhibition of feeding by intracerebral calcitonin mimics the effects of peripheral injections, in a dosage range that is on the order of the proportion of the weight ratio of brain to total body mass (ii) The dosages of intracerebral calcitonin that inhibit feeding are smaller than the minimum dosages of most other peptide hormones of extra-neuronal origin , such as ACTH, angiotensin II, and the enkephalins, that have previously been shown to induce behavioral alterations when administered into rat cerebral ventricles; (iii) Inactivation of calcitonin by treatments expected to alter the characteristics of calcitonin as a peptide eliminated the ability of calcitonin to inhibit feeding; (iv) such evidence as we have does not indicate that calcitonin inhibits feeding by producing illness or debilitation. It is therefore tempting to speculate that endogenous calcitonin is involved in the regulation of feeding and appetite, perhaps in certain specific situations, such as in infants, during lactation, or in calcium-specific hunger. However, further research will be necessary to determine whether the effects of calcitonin on feeding are merely pharmacological, or whether they mimic an effect of endogenously-secreted calcitonin.

XX. HLA Antigens

A survey of 14 published studies found no consistent association between HLA antigens and schizophrenia. Since the studies lacked both a shared diagnostic system or biological criteria, an investigation was undertaken using Research Diagnostic Criteria and CT scan findings. Typing for HLA antigens at loci A, B and C was carried out on 126 patients. Among 64 Black schizophrenic patients there was an increase of HLA-A_2 that remained significant even after correcting for the number of antigens studied. When the patients for whom CT scans were available was divided according to the presence or absence of evidence of brain atrophy there was an increase of A_2 with black schizophrenic patients without atrophy (70% vs 30) that remained significant after multiplying by the number of antigens studied but there was no significant increase of A_2 on those with atrophy. Similar trends held for the white population but they failed to reach significance. The need for HLA studies on biological defined groups of schizophrenics is stressed.

Several diseases have been shown to be associated with specific human leukocyte antigens. While there have been a number of reported associations between the HLA system and schizophrenia, none of these has been consistent. This lack of consistency could be for a number of reasons including: 1) differences in diagnostic systems and 2) biological differences among schizophrenics.

XXI. Laterality

On the basis of a study of handedness in monozygotic (MZ) twins at least one of whom was schizophrenic we hypothesized the existence of a subgroup of schizophrenic patients characterized by anomalous lateralization and a milder form of illness. To test this hypothesis we examined laterality in a group of schizophrenic patients.

Our sample consisted of 66 in-patients at Saint Elizabeths Hospital, Washington, D.C. who met Research Diagnostic Criteria (RDC) for schizophrenia or schizo-affective psychosis. The schizophrenic patients were further subdiagnosed into chronic (present episode of at least one year duration or incomplete recovery from previous episodes) and acute (present episode less than one year or complete recovery from previous episodes) by a rater unaware of the patients laterality. The patients were tested for laterality on a 12 item test. Those who used their right eye, hand or foot on 9 or more items were considered right handed, the others were considered not right handed (left handed or ambidextrous). Also a subgroup of 55 were given the Torque Test which consists of drawing 3 circles with each hand. Those who drew a circle in the clockwise direction with either hand were said to have Torque. Showing Torque is thought to be a left-handed characteristic and in behaviorally disturbed children has been shown to be associated with a figure diagnosis of schizophrenia.

The results indicate that anomalous lateralization, as determined either by a battery of tasks or by the Torque test, appears to be associated with a milder form of schizophrenia. This is similar to the findings of Lishman and McMeekan who found an increase in left handedness in schizo-affective psychoses but not "true" schizophrenia. It is also in keeping with our hypothesis that there exists a subgroup of schizophrenic patients with a milder form of illness who show anomalous lateralization. As a corollary, these results suggest that in severely ill schizophrenics laterality disturbances may not be more prevalent than in the general population.

If our interpretation is correct it has several implications. First, it suggests that the proportion of left handedness in a group of schizophrenics will vary depending on the percentage of milder forms of schizophrenias included in this sample. It is also possible that anomalous laterilization is important in the etiology of some schizophrenias. A study of MZ twins with schizophrenia shows that in twinships with at least one left handed twin the left handers tend to suffer from a mild schizophrenia and the right handers are not schizophrenic. This suggests that left handedness may, in some individuals predispose to schizophrenia. Also reversed neuroanatomical asymmetries on computed tomography scans, (another measure of anomalous lateralization) have been found with increased frequency only in those schizophrenic patients who show no evidence of brain atrophy. This supports the notion of etiological heterogeneity of schizophrenia and the possibility that anomalous laterialization is etiologically significant in some schizophrenic patients.

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OTHER:	Ivan Waldman	Psychologist	SMRC

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ongoing collection of daily behavior of 8 schizophrenic patients from WAW-4. Purpose is to explore evidence for homeostatic regulation in such data and to characterize such regulation mathematically and seek in basis of differentiating (1) clinical groups and (2) changes occurring with treatment chages in individual patients. Controlling hypothesis is that behavioral steady states are more difficult for schizophrenics to achieve and our basis of his progressive withdrawal. Data suggests hebephrenics exhibit most marked behavioral cycles. Focus will be on comparing effects on varying psychotropic drug dosage on time-series parameters (phenothiazines and lithium). Review of past data confirms spectrum peaks all hebephrenic records, none for paranoid and catatonic patients. 6-variable theoretical schemes covers different wave-forms observed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To obtain a quantitative description of behavior which permits quantitative comparison of a variety of theoretical models. Governing the regulation of higher common excitatory states.

Methods Employed:

Behavioral notes are made 60 times daily on each of 8 chronic schizophrenic patients on Ward 4 of the William A. White Building of Saint Elizabeths Hospital. The unit datum is a percentage of daily (or weekly) checktimes positive for a given behavior (e.g., pacing, finger movement, talking, etc.). The resulting time-series is examined in a variety of ways to evidence for non-random components. Stimulation techniques utilizing differential equations are being applied to the data.

Major Findings:

Behavioral cycles have been identified in 5 of the patients on Ward 4, using Power spectra and auto-regressive schemes.

Preliminary data suggest that lithium eliminates a six-day cycle in two patients, modifies it in one patient. Librium has an additional flattening effect on one five-day cycle when added to lithium.

Significance to Bio-Medical Research and the Program of the Institute:

While the basic purpose of this study continues to be the ultimate development of inputoutput modeling of human behavior in specific environments, with particular reference to a study of disturbances of regulation in the major psychoses, it would appear that the overall methodology may be applicable to the study of response to psychotropic drugs, and perhaps yield some clues as to their mode of action in functional as opposed to biochemical terms. Overall strategy controlled by the hypothesis of a homeostatis deficit in schizophrenia. Hebephrenia seems biologically separable on the basis of current data, and supports concept of central homeostatic failure in these patients.

Proposed Course:

Continuation of (1) data collection; (2) theoretical modeling; and (3) evaluation of drug responses. Current strategy is to use theoretical modeling with differential equations on all long data stretches with stable medication regimes, and examine parameter changes due to medication and/or environmental influences (e.g., participation in new treatment program without drug change.)

Publications:

1. Reynolds, T.D., London, W., and York, J.: Behavioral rhythms in schizophrenia. J. Nerv. Ment. Dis. 166: 489-499, 1978.

2. Reynolds, T.D.: Phase Plane Description of Schizophrenic Behavior Rhythms. in Biological Psychiatry Today; P. 288-293, Elsevier, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01337-09 SMRC

PERIOD COVERED
October 1, 1979 to September 30, 1980.

TITLE OF PROJECT (80 characters or less)

Studies on Drugs of Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.J. Wyatt, Chief, Lab. of Clin. Psychopharmacology, R.C. Stillman, Research Psychiatrist, NIDA and Univ. of Calif.; E.V. DeRenzo, Psychologist, SMRC; E. Parker, Psychologist, NIAAA; J.C. Gillin, Deputy Chief, Lab. of Clin. Psychopharmacology, SMRC; W.B. Mendelson, Staff Psychiatrist, SMRC; D. Stoff, Psychologist, SMRC; W. Freed, Staff Fellow, SMRC; R. J. Wagner, Staff Psychiatrist, SMRC; F. Karoum, Chemist, SMRC; J.E. Kleinman, Staff Psychiatrist, SMRC; OTHER: H. Weingartner, Psychiatrist, DCBR; W. Bunney, Director, BPB.

COOPERATING UNITS (if any)
Division of Research, National Institute of Drug Abuse; Laboratory of Clinical Science, National Institute of Mental Health; National Institute of Mental Health.

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	4	4

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SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project continues to be determination of the mechanism of actions of drugs of abuse (particularly marijuana and alcohol) and the reasons by these drugs are used. We are also interested in ways that the understanding of drug effects can help us understand normal mental function or mental illness.

Project Description:

Objectives:

To further our understanding of the nature and action of pharmacologic agents which are classified as drugs of abuse.

Methods Employed:

Clinical studies and animal models shed light on the nature and action of these drugs. The methods employed will be discussed under each class of drugs.

Marijuana and Alcohol:

Understanding the similarities and differences between marijuana and alcohol, the two most widely used social intoxicants, is essential. Previously, investigators had largely assumed that complex dose-response curves were needed for each effect to be compared; for example, a decrement in free recall produced by one dose of alcohol and not by another of marijuana might not indicate any real difference between the two, but might show that the doses were not comparable; i.e., that by giving more marijuana the free recall might be equally affected. Our approach has been to look at a profile of effects of each drug at a given dose, one which users themselves report as effective. We then look for "crossovers" in which, at these two somewhat arbitary doses (one for each drug), there is greater impairment for task A by alcohol and greater impairment for task B by marijuana. When these are formed, we can say that alcohol "disrupts" A more than marijuana whose effects on B are greater.

Major Findings:

Such crossovers have been demonstrated in two studies: an incidental/intentional memory study and a study of eye movement and memory. In the former, marijuana (15 mg THC smoked) produced significantly more intrusions than did alcohol (1.0 mg/kg); however, alcohol, at the same dose disrupted free recall significantly more than marijuana the aforementioned dose. This is clearly of significance in understanding the differential effects of the two drugs (and perhaps their different appeal). Marijuana produces "extra" recall items, alcohol fewer--both are deleterious to function, but presumably involve different mechanisms. Put another way, marijuana does not impair ability to access items, but rather impairs the ability to discriminate those items which were originally present. Alcohol, on the other hand, impairs the access to these items, but not the ability to discriminate that which had in fact been previously encountered.

A similar trend was found in a study of eye-movements and visual memory. Alcohol impaired memory for "token changes" (in which an object was replaced by one quite unlike it) and "type changes" (in which an object was replaced by one quite related to it). Marijuana, on the other hand, showed no significant effect on these types of recall but did diminish subjects ability to detect rearrangements, which alcohol did not interfere with. Thus, alcohol interfered with recall of presence or absence of objects, marijuana with recall of their special arrangement.

In two experiments with normal male subjects, subsequent memory was significantly improved with the injestion of alcohol (1 ml/kg) immediately after learning. Marijuana, however, (at 15 mg) had no significant postacquisition effect. Resolving and characterizing such differences is important not only in understanding the effects of the drugs but also in understanding which components of memory are themselves separable pharmacologically. The facilitation of visual and verbal memory with alcohol has implications for the interference and consolidation views of memory. A final difference between the drugs is a rate of visual scanning. Marijuana accelerates the rate of visual scanning (number of eye fixations per second) while alcohol retards it.

A surprising correlation (0.8 or higher) has been found in subjects' ratings of overall intoxication level using alcohol one day and marijuana the other. Subjects who report themselves little intoxicated by alcohol are the same ones who report themselves little intoxicated by marijuana. (This is in contrast to popular assumptions that susceptibility for alcohol is pretty much independent of susceptibility to marijuana.) Correlations were not significant between subjective ratings of intoxication and objective measures (e.g., blood alcohol level or change in pulse rate), nor between objective measures themselves.

Hemispheric Differences

We are investigating hemispheric specialization which we discovered by comparison of marijuana and alcohol effects on hemispheric function. This is the first time any group has found a reliable way to produce hemispheric differences in the laboratory. It involves presentations of a two-choice task which becomes more and more differentiated to separate hemispheres over several thousand trials. The method is quite powerful and will be of great value when it is sufficiently refined to reapply to the drug studies from which it serendipitously evolved.

Hypnotics

In April 1979, the Institute of Medicine, National Academy of Sciences, issued its report, Sleeping Pills, Medical Practice, and Insomnia, the result of a study lasting over one year. Dr. J. Christian Gillin was a member of the Steering Committee, and Dr. Wallace Mendelson was the chief technical consultant. Dr. Wyatt also advised the committee. This study was inspired by NIDA's previously published report. It drew attention to some of the less publicized unanswered questions and hazards associated with long-term use of benzodiazepines, such as interaction with alcohol, accumulation of long-term metabolites, adverse effects in the elderly, and the indications for hypnotic use. As a result of his participation in this study, Dr. Mendelson has written a book, The Dose and Measure of Sleeping Pills, to be published shortly by Plenum Press. The Laboratory has had a long interest in developing less dangerous, more efficacious pharmacological treatments for insomnia. Wyatt *et al.* (Lancet, 1970, 842-846) were among the first to suggest that L-tryptophan functions as a "natural hypnotic." Within the last year, we have also studied the effect of the four major "hypnotoxins" in the rat: Pappenheimer's Substance S, Veonnier's DSIP, Peve's arginine vasotoxin (AVT), and Nagasacki's extract from sleep deprived brain. We have not been able to demonstrate an hypnotic effect for any of these substances.

In December 1979, the Surgeon General initiated Project Sleep, a three-year joint public-private effort to upgrade the quality of clinical care provided to patients with clinical sleep disorders. It will be primarily an educational program aimed at the health professions. Drs. Gillin and Mendelson are members of the Steering Committee, which is chaired by Dr. Charles Krauthamer, Chief of Dr. Klerman's Division of Science.

Opiate Antagonists and Agonists

Basic Studies

Turnover and metabolism of catecholamines in rats injected with 10 (low dose) or 40 (high dose) mg/kg morphine sulfate i.p. were evaluated in brain, spinal cord regions, heart, celiac and superior cervical ganglia. This study was primarily undertaken to characterize whether opiate receptors modulate the metabolism of catecholamines in ganglia. Comparisons were made with other tissues. To eliminate the influence of opiate peptides secreted by adrenal medulla and CNS and of opiate receptors (autoreceptors) located on afferent nerves, on the ganglionic responses evoked by morphine, we used demedullated rats with unilaterally decentralized superior cervical ganglia.

Demedullation failed to alter dopamine turnover or metabolism in the ganglia, but significantly increased norepinephrine concentration and turnover in both sham operated and decentralized superior cervical ganglia but not in the celiac ganglia. Provisionally, the latter effect in the superior cervical ganglia was attributed to the absence in the blood of modulatory opiate peptide which normally are secreted by the adrenal medulla. Both low and high doses of morphine failed to alter the turnover jof heart and sympathetic ganglia norepinephrine. The changes in norepinephrine turnover observed in the spinal cord and brain were variable. Low doses of morphine only increased dopamine metabolism in the caudate. High doses of morphine significantly increased dopamine turnover and metabolism in the cervical and superior cervical ganglia, as well as in the caudate and ventral or dorsal horn of the spinal cord of demedullated rats. High doses of morpine increased the dihydroxyphenylacetc acid (DOPAC) content of sympathetic ganglia, naloxone inhibited this change suggesting that opiate receptors may mediate this action. The superior cervical ganglia of normal (intact medulla) rats is resistant to high doses of morphine.

Anticholinergic drugs (atropine and hexamethonium) failed to antagonize the increase in DOPAC content of superior cervical ganglia elicited by the high dose of morphine, suggesting that this increase was not mediated via preganglionic cholinergic nerves. A dissociation between cholinergic and opiate receptor mediation is suggested by the failure of naloxone to antagonize the increase in ganglionic DOPAC produced by oxotremorine (0.2 mg/kg, subcutaneously).

The results reported reveal some basic and comparative information on the turnover rate and steady-state concentration of catecholamines in various central and peripheral tissues (Karoum et al.).

Clinical Studies

Naloxone has been reported to reduce auditory hallucinations and thought disorder in chronic schizophrenic patients. We have studied this by examining changes in psychiatric symptomatology of schizophrenics after naloxone versus saline infusion. In an initial study, 0.4 mg of naloxone was infused in five patients. This dosage produced a subclinical decrease in the abnormal thought content item on the Brief Psychiatric Rating Scale (BPRS). In subsequent studies, the interaction of antipsychotic medication with naloxone in chronic schizophrenic patients has been explored.

As of now, 22 patients diagnosed as chronic schizophrenic using Research Diagnostic Criteria (RDC) have had infusions of 15 mg of naloxone (double-blind with placebo). In the drug-free state (as opposed to drug-free affective patients or control subjects), the schizophrenic group showed plasma growth hormone and prolactin elevations from 15 through 60 minutes (Kleinman, 1980). When this patient group was studied while receiving antipsychotic medication, no prolactin increased elevation was observed, but, a delayed growth hormone rise (45 through 120 minutes) occurred. Of note, a subclinical abnormal thought content decrease was seen in the currently medicated patient group. Moreover, a few medicated patients demonstrated a significant clinical diminution in auditory hallucinations. One patient in particular could identify the exact time the last hallucination occurred (two hours after infusion which lasted up to six hours). We have interest in following up this subgroup with repeated double-blind infusions. If promising results continue, oral naltrexone would be given for more chronic maintenance.

Evidence has accumulated that opiate agonists also may serve a role in relation to psychosis. Following up promising leads, we are studying beta-endorphin for therapeutic efficacy. As of now, we have studied two patients. In one, no significant benefit occurred; in the other, only repeated (three daily) infusions seemed to produce some decrease in symptomatology additively. In all drug-free schizophrenic patients, plasma prolactin increased subsequent to infusions. Of future interest will be those patients who benefit from repeated administration. A methadone (intravenous and oral) protocol is submitted to test this more prolonged beneficial response. As in all opiate agonist research, possibility for any dependency is minimized by a short-term, strictly controlled protocol.

Since joining the Laboratory, Dr. David Stoff has been the principal investigator in animal studies of abused drugs. During the past year, Dr. Stoff finished testing the chronic activity monitoring system which will be used for the analysis of chronic drug use in small animals.

We have evaluated the animal models which have been used for drug-induced hallucinations and proposed criteria which should be met by an "ideal" animal model (Stoff et al., 1968). Our data suggest that some of the behavioral disturbances in animals caused by amphetamines and hallucinogens can be explained by a deficit in attention and/or information processing (Stoff et al., 1978). We have also discussed the interaction of hallucinogens with other drugs (Wyatt et al., 1976), the role of indole hallucinogens in schizophrenia (Gillin and Wyatt, 1977) and the relevance of PEA to drugs of abuse and neuropsychiatric disorders (Wyatt et al., 1977).

In this country, there has been a steady increase in the nonmedical popularity of CNS stimulants (amphetamine-related compounds, cocaine) and hallucinogens (phencyclidine, LSD) over the last decade. A parallel increase in laboratory research aimed at elucidating the mechanism of action of these drugs and developing effective treatments is evidenced by the wealth of data in the literature which describe the physiological, pharmacological, behavioral and biochemical actions of psychomotor stimulants and hallucinogens in a wide variety of preparations. However, most of this work has been considered primarily with characterizing the acute effects of these drugs, although it is the chronic state of intoxication which, clinically speaking, leads to psychosis and movement disorders. Chronic amphetamine intoxication is an insidious problem in the majority of cases--symptoms usually take a long time to appear and daily intake of drugs by chronic abusers can become incredibly high before significant problems appear. Eventually, however, the drug abuser suffers personality changes, weight losses, and marked impairment of cardiovascular, central nervous, and gastrointestinal systems.

Information is gathering to suggest that chronic use of some CNS stimulants and some hallucinogenic agents leads to more exaggerated catecholaminergic effects than single administration (instead of tolerance as is the case with the CNS depression; i.e., barbiturates, alcohol, benzodiazepines and the narcotic analgesics). This is based on animal experiments after chronic drug exposure using electrophysiological (i.e., Post, R.M. and Kopanda, R.T.: Am. J. Psychiatry 133:627-637, 1976), behavioral (e.g., Klawans, H.L. and Margolin, D.L.: Arch. Gen. Psychiatry 32:723-732, 1975), and neurochemical measurements (e.g., Ellison, G., Eison, M.S., Huberman, H.S. and Daniel, F.: Science 201:276-278, 1978). These data bear out the growing concern about the psychological (not physical) dependence engendered by the chronic use of these drugs and possibility of the structural damage caused by continuous drug exposure.

It is well known that psychoactive drugs have a variety of psychological effects: simply put, both "wanted and unwanted". These effects have been referred to as "euphoria," "good trip," psychedelic," eidetics" versus "dysphoria," "bad trip," "psychotomimetic," "psychotoxic." These different effects may work through different mechanisms, involve different neural substrates, occur under different environmental conditions, and require different treatment modalities. Because the pleasant effects (i.e., "relaxed" alertness, increased energy, euphoria--for stimulants; perceptual changes, transcendental state--for hallucinogens) usually occur at relatively low doses after acute administration, there is a tendency (which can become a "craving") to use higher doses and/or more frequent drug administrations in order to intensify the effects. However, the euphoria is rapidly replaced by psychological depression, motivating users to repeat the dose very frequently in an attempt to maintain a continuous "high." The individual is then caught in the bind of continually taking the drugs to presumably relieve the state of tension or dysphoria--when the original motivation was to continue drug-taking to get a better euphoria. Because chronic stimulant use is often accompanied by the development of "reverse tolerance," with increasing sensitivity to the drug's effects, this in turn may induce excitatory symptoms (at some doses) and prompt the development of "reverse tolerance," with increasing sensitivity to the drug's effects, this in turn may induce excitatory symptoms (at some doses) and prompt the development of agitation, anxiety, paranoid thoughts, auditory and visual hallucinations and, at times, violent irrational behavior. Furthermore, repeated drug use may change the response to subsequent drug administration. With long-term use, tolerance does not develop to the dysphoric and/or psychosis-inducing effects and chronic users either experience less euphoria or become more sensitive to the dysphoric and/or psychotomimetic effects. One might speculate that sensitization causes a shift on the dose response continum so that a previous euphoriant dose becomes dysphoriant.

Due to our interest in the chronic state of intoxication in humans, we decided to construct a "model" behavioral system for small animals which: (1) continuously monitored every aspect of the laboratory animal's behavior without any intrusive techniques in order to get the animal's life history. We focused on behavioral parameters which bear some relevance to the chronic stimulant and hallucinogenic drug states and show some change in response to chronic treatment; (2) provides both a home and experimental situation to mimic the laboratory animal's habitat as much as possible; (3) easily allowed for continuous drug administration without any intervention and the simultaneous monitoring of ongoing behavioral changes (4) has been reasonably well characterized as an experimental situation making it possible to ask some relevant conceptual questions.

The Activity System

We have developed an activity system which is a more precise, objective and practical technique than traditional observational methods in measuring drug-induced motor changes. The level of behavioral complexity which can be monitored is limited only by our ability to define the phenomena.

The activity system is schematically diagrammed in Figures 1 and 2. The animals live in a square plexiglass cage with access to food and water. This cage is housed in a shielded environmental enclosure which provides ventilation and lighting. Conductive surfaces which are mounted outside and parallel to the plane of each plexiglass wall operate with the grounded stainless steel rod floor to generate four functionally electric fields inside the cage. The animal's location in the cage is transduced by the four physically overlapping but functionally separate electric fields within the cage. The animal distorts each field according to his proximity to each plate thus affecting the value of each capacitance. Electronic circuitry generates from these values two analog voltages which represent the animal's location along x-y axis of the cage floor (Fig. 3). A microprocessor transforms these signals into linear values which are processed by a program (for a gross movement and rearing algorithm), reflecting the position of the animal and occurrence of rearing. The program is burnt on a permanent read-only memory in this minicomputer to recognize the record activity information. This information is displayed, in digital form, on a terminal and is also stored on a tape cassette. The information on cassette can then be transferred to a PDP 11-34 which prints out a hard copy of the data and does the appropriate statistical analyses.

Figures 4 to 6 show the analog voltages and the associated digital output for three specific behaviors: rears (rear threshold=2500 mv), gross movements (gross movement threshold=100 mm), and fine movements (fine movement threshold=5 mm) measured continuously for 60 minutes after an injection of d-amphetamine (6 mg/kg, i.p.). Figure 4 shows random hyperactivity with rearing at 5 minutes (note, two rears) leading to more repetitive hyperactivity with more rearing at 10 minutes (note, six rears). By 20 minutes, rearing is absent and by 30 minutes, there is repetitive circling around the cage (note, rhythmic waves) (see Fig. 5). By 43 minutes, behavior is more restricted (note, small amplitude waves) and by 50 minutes movements are in place, restricted and repetitive (note, small amplitude waves only in SE quadrant) (see Figure 6). These data represent the time-dependent qualitative shifts in amphetamine-induced behavior from generalized excitement to repetitive hyperactivity to repetitive restricted movements.

Figure 7 shows the normal rat's circadian rhythm in activity at a low movement threshold of 10 mm, and Fig. 8 shows the ultradian rhythm of chronic haloperidol (up to 15 weeks) on rearing behavior.

Shuttlebox Avoidance

We have conducted a series of investigations in rats of the effects of hallucinogenic drugs in the shuttlebox paradigm. We report that both LSD and mescaline have two distinct opposite effects at the same doses (Stoff *et al.*, 1974) and postulated that one of these effects is analogous to the "good trip" in humans and the other is analogous to the "bad trip." The two distinct effects produced by LSD and mescaline on shuttlebox responding are: (1) Excitation or facilitation of responding and (2) inhibition or disruption of responding. The presence of stress is common to both excitation in rats and "bad trip" effects in man, whereas the relative absence of stress is common to both disruption in rats and "good trip" effects in man. Excitation in rats may parallel the "bad trip" effects in man because excitation occurs in a relatively stressful situation and likewise, the "bad trip" effects in man occur in patients who present themselves in hospital emergency rooms with acute psychotic disturbance and emotional stress associated with drug intake. Similarly, disruption in rats may parallel the "good trip" effects in man since disruption occurs in a relatively nonstressful situation and likewise, the "good trip" effects in man occur in a pleasant and supportive environment.

Lesion-Rotation Model

Chronic exposure to dopamine agonists occurs in two clinical situations: chronic amphetamine abuse and the use of levo-dopa in the treatment of parkinsonism. In both situations, psychiatric disorders and abnormal movements are frequently observed. The supersensitivity phenomenon may be an important factor in the stimulant psychoses and in the induction of dyskinesia in chronic amphetamine users. Ungerstedt and Arbuthnott (Brain Res. 24:485-493,1970) developed a quantitative animal model which has been useful in assessing the potential therapeutic value of drugs in the treatment of parkinsonism. This model may prove useful in developing treatments for drugs which stimulate dopamine (like amphetamine or phencyclidine) because it provides a way of telling whether the drug is acting pre- or post-synaptically. Such pharmacological dissection sheds light on the drug of choice for treatment. It has been shown that drugs which indirectly stimulate dopamine receptors produce turning behavior in unilaterally lesioned (via 6-hydroxydopamine) toward the lesioned side (i.e., ipsilateral rotation) and drugs which directly stimulate dopamine receptors produce turning behavior towards the unlesioned side (i.e., contralateral rotation).

1. Speed: Amphetamine

Chronic amphetamine-induced behavioral changes in animals are invariably accompanied by motor stereotypies which are species-specific. The significant characteristic of these behavior patterns is the compulsive way in which they are expressed, to the exclusion of other behaviors which are normally present, such as grooming, exploration or feeding. The fact that the particular limited response is performed repeatedly, often rhythmically and apparently without purpose, characterizes it as "stereotaxic." An extensive and systematic series of studies utilizing such techniques as selective enzyme inhibition, agonist-antagonist relationships, and local microinjections have documented the importance of central dopaminergic facilitation (particularly in the striatum) in mediating this stereotyped behavior. These studies led to the development of the concept that drug-induced stereotyped behavior in animals might serve as a model for stimulant psychosis in humans, a well-known side effect of chronic amphetamine abuse. The persistent stereotypy in chronic amphetamine addicts has been observed (e.g., Garver, D.L., Schlemmer, R.F., Mass, J.W., Davis, J.M., Am. J. Psychiatry 132:33-38, 1975). It has been referred to as "punding" by Rylander, G. (in: Abuse of Central Stimulants, F. Sjoquist and M. Tottie, eds., Raven Press, New York, 1969, pp. 251-273), who noted that Swedish Preludin (phenmetrazine) addicts were preoccupied with useless, repetitive, minute tasks.

Recent Results

Genetic differences in behavioral response to d-amphetamine have been reported in mice (Satinder et al., J. Comp. Physiol. Psychol., 71:443-447, 1970) and rats (Segal et al., Science 189:301-303, 1975). We have been studying behavioral responses of a number of inbred strains of mice to d-amphetamine and to haloperidol followed 30 minutes later by d-amphetamine. We have tested 11 strains of mice, including two parent strains (C57BL/6By and BALB/cBy), two F-1 hybrids (CB6F1 and B6CF1), and seven recombinant inbred strains (CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ and CXBK). We have studied the stereotypy and locomotor responses of these strains to 5 mg/kg d-amphetamine alone as well as to the same amount of the drug given 30 minutes after an injection of 0.1 mg/kg haloperidol. The tests with inbred strains of mice are useful for delineating genetic transmission of responsiveness to drugs. Our results so far have interesting implications for understanding possible hereditary transmission of responses to d-amphetamine and haloperidol (Jeste et al., unpublished data).

2. The Natural Speed: Phenylethylamine (PEA)

PEA is almost structurally identical to amphetamine and has very similar pharmacological, physiological and behavioral actions to amphetamine. Since PEA is amphetamine-like, but is naturally present (Inwang et al., 1973; Nakejima et al., 1964; Saavedra, 1964), whereas amphetamine is not, PEA may serve as a useful animal model to study chronic stimulant abuse and the accompanying residual effects. Several authors have emphasized the existence of residual psychotic symptomatology and the possibility of an altered state of physiology following chronic amphetamine use (e.g., Angrist, B.M., et al., Int. Pharmacopsychiatry 2:125-139, 1969; Utena, H., Prog. Brain Res. 21:199-207, 1966). The psychoses which outlast the presence of amphetamine in urine occur for several months following the cessation of amphetamine use. The persistent psychotic states may be due to the continued presence of a naturally-occurring amphetamine such as PEA.

In a series of experiments, we have shown that PEA has amphetamine-like behavioral effects when monoamine oxidase is inhibited (Moja et al., 1976), these effects are antagonized by dopamine receptor blockers (Moja et al., 1978) and testosterone (Freemark et al., 1978), and chronic neuroleptics cause supersensitivity to PEA (Stoff et al., 1978). Tolerance develops to PEA's effects on learned behavior, but not to PEA-induced stereotypy (Stoff et al., 1978). We found that two other compounds have similar behavioral effects to amphetamine and PEA: paramethoxyphenylethylamine, a derivative of PEA (Cannon et al., 1978) and amfonelic acid, a specific central dopaminergic stimulant (Jeste et al., 1978). The serotonergic system appears to be involved in the behavioral stimulating effects of mescaline (Stoff et al., 1976) and amphetamine (Cannon et al., 1976).

Recent Results

The repeated administration of PEA to laboratory animals produces neither tolerance nor physical dependence. "Reverse tolerance" has been used to describe the increased susceptibility to the stimulant effects of PEA.

As a model of the persistent effects of stimulants with long-term use, we have studied the effects of chronic PEA (600 mg/kg, 30 times. p.o.) to rats on behavior, tyrosine hydroxylase activity, and GABA binding (Stoff et al.) Our data show that chronic PEA causes time-dependent behavioral changes together with an increase in the Vmax of caudate tyrosine hydroxylase (results on GABA are not yet tabulated). These effects outlast the duration of drug treatment, eliminating drug dispositional and metabolic factors. In addition, these effects do not appear to depend upon dopamine receptor activation since they are not blocked by concurrent chronic haloperidol administration.

Catalepsy is the pharmacological converse of stereotypy. Drugs which induce catalepsy are capable of antagonizing stereotyped behavior. As a model of protection against the stimulant psychoses, we have studied whether CNS stimulants reverse chlorpromazine's catalepsy and allosteric activation of caudate tyrosine hydroxylase (TH). We found that PEA, amphetamine, and apomorphine cause dose-dependent reversal of both CPZ-catalepsy and TH activation (Stoff et al.)

3. The Businessman's Trip: N,N-Dimethyltryptamine (DMT)

Because DMT is a short-acting hallucinogen, it gained the reputation of its nickname as a reprieve from the hard working day (Szara, 1956). DMT is found in some human tissue (Walker et al., 1973) and enzymes for producing it are present in human lung (Mandel et al., 1972) and may be present in human brain and blood as well (Mandell and Morgan, 1971; Saavedra and Axelrod, 1972; Wyatt et al., 1973). We found that when DMT is given to man it produces an intense psychological disturbance (Kaplan et al., 1974). Because of the brevity of its effects and the intensity of its reaction, it has become well known in the drug subculture.

DMT causes dose response disruption of shuttlebox avoidance (Stoff et al., 1977) which can be blocked by chronic neuroleptic treatment (Stoff et al., 1978). Five-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), a close relative of DMT, has hallucinogenic properties more powerful than DMT (Shulgin, 1970). Five-MeO-DMT is more potent than DMT is disrupting shuttlebox behavior (Stoff et al., 1978). Both DMT and

5-MeO-DMT produce highly toxic effects in several animal species (lethal in sheep) (Gillin et al., 1976). Because of its use in the drug subculture (Legal High, Annon, 1973) and the toxicity of its effects, it is important to develop a reliable screening device for this drug.

Recent Results

To discover an effective antidote to DMT, we have conducted an extensive series of drug interaction experiments using the DMT shuttlebox disruption paradigm. DMT-disruption is not blocked by acute or chronic pretreatment with drugs which inhibit serotonin (i.e., methysergide, methiothepin, BC-105, cyproheptadine), dopamine (i.e., haloperidol, pimozide, chlorpromazine, thioridazine or norepinephrine--i.e., propranolol). These data provide evidence against a monoaminergic mechanism of DMT suggesting that DMT acts nonspecifically or through its own, as yet undiscovered, receptor.

4. Angel Dust: Phencyclidine (PCP)

Misrepresentation of PCP is widespread because of its ready availability. Users are often led to believe that they are being sold more exotic natural drugs, such as mescaline, when in fact the pills are entirely PCP. Such deception makes it difficult to make an accurate diagnosis and provide the best treatment. Individuals who have taken PCP do not describe the increased ability for self-observation or kaleidoscopic visual hallucinations reported by users of the more classic hallucinogens (Schowalter et al., 1977, Am. J. Psychiatry 134:1234, 1977). On the contrary, the phencyclidine experience can be unpleasant with impaired thought, disorientation, delirium and marked anxiety. There is some concern that PCP intoxication leads to behavioral changes persisting for days or weeks after drug use (NIDA Conference on Phencyclidine, Asilomar, California, February 1978).

We have tried to develop an animal model for persistent effects of PCP. We found that PCP facilitates acquisition of shuttlebox avoidance and that this effect may last at least 24 hours after drug injection (Stoff et al., 1978). Further, this effect may be state-dependent which has been suggested as a predictor of the abuse potential of drugs (Overton, D.A. and Batta, S.K., In: Predicting Dependence Liability of Stimulant and Depressant Drugs, University Park Press, Baltimore, 1977, pp. 125-137.

Phencyclidine is known to produce a dose-related stimulant of locomotor activity in mice (Chen et al., J. Pharmacol. Exp. Ther., 127:241-250, 1959), similar to the activation produced by amphetamine. We have used this property of PCP as an animal model to screen for agents capable of blocking behavioral effects of PCP (Freed, 1979). Because many drugs influence the locomotor activity of animals, PCP-induced stimulation was evaluated in relationship to the effects of the drugs on the locomotor activity in the absence of PCP. Drugs which were found to be capable of completely blocking this effect of PCP included chlorpromazine, clozapine, muscimol, imidazole-4-acetic acid, and methylsergide in high doses. Baclofen, cyproheptadine, haloperidol, and yohimbine were partially effective. Many other agents, such as naloxone, diazepam, cinnanserin, propranolol, benztropine, arecoline, pilocarine, reserpine, alpha-methyl--

para-tyrosine, phentolamine, diphehydramine, and cloral hydrate were either ineffective or enhanced effects of PCP. Neurochemical studies suggest that PCP interacts primarily with brain cholinergic and opiate receptors (Vincent et al., Brain Res. 152:176-182, 1978; Maayani et al., 23:1263-1281, 1974), although effect on metabolism of serotonin and norepinephrine have also been described (Leonard and Tonge, Life Sci., 8:815-825, 1969). The present results suggest that manipulations of brain dopamine, serotonin, and gamma-aminobutyric acid are most effective in blocking PCP-induced behavioral alterations. It is therefore concluded that studies of neurotransmitter binding and metabolism are not good predictors of the neuropharmacology of PCP-induced alterations in behavior.

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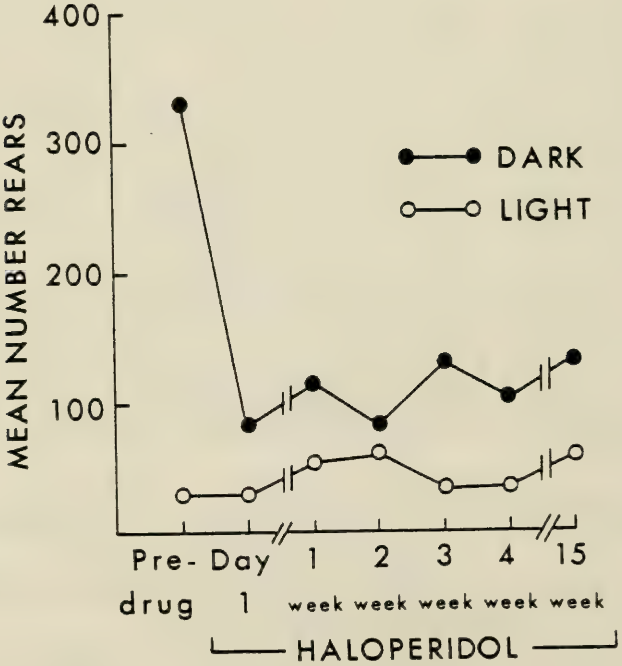
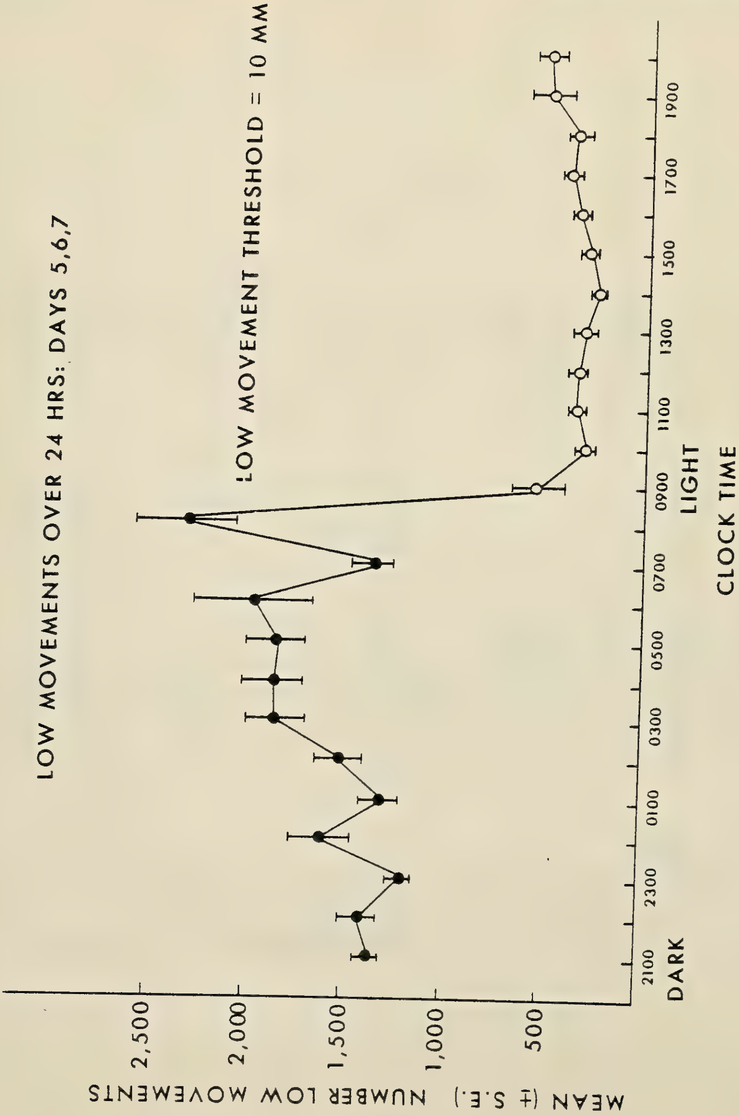


EFFECTS OF CHRONIC HALOPERIDOL ON REARS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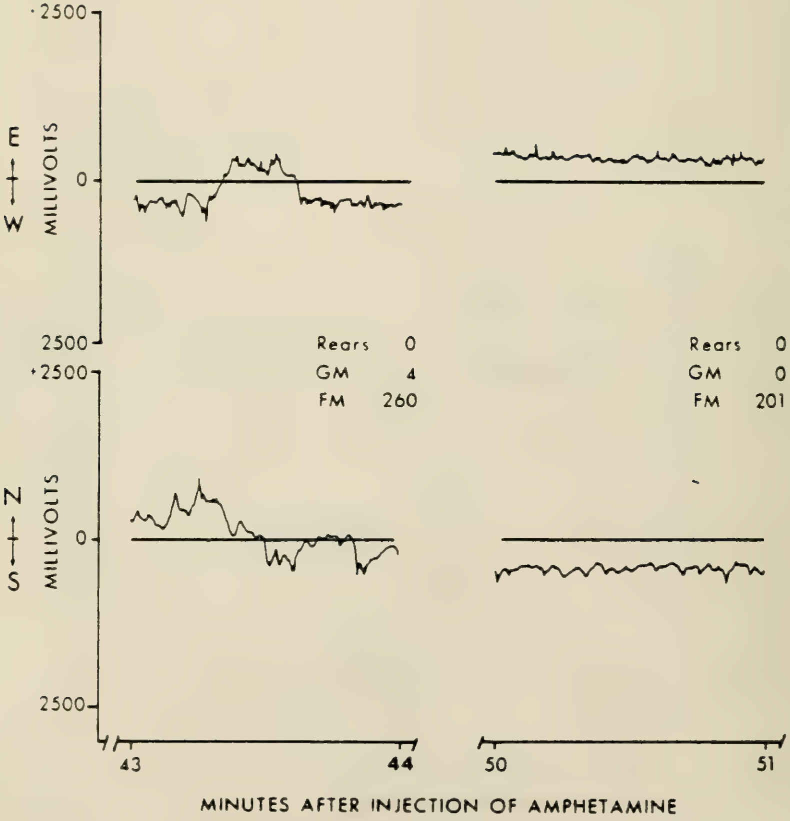
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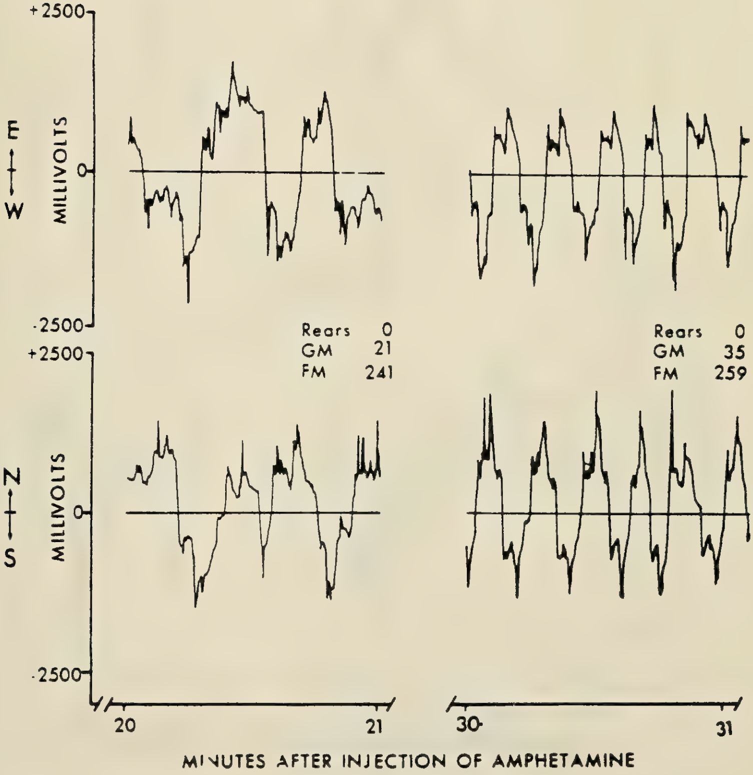
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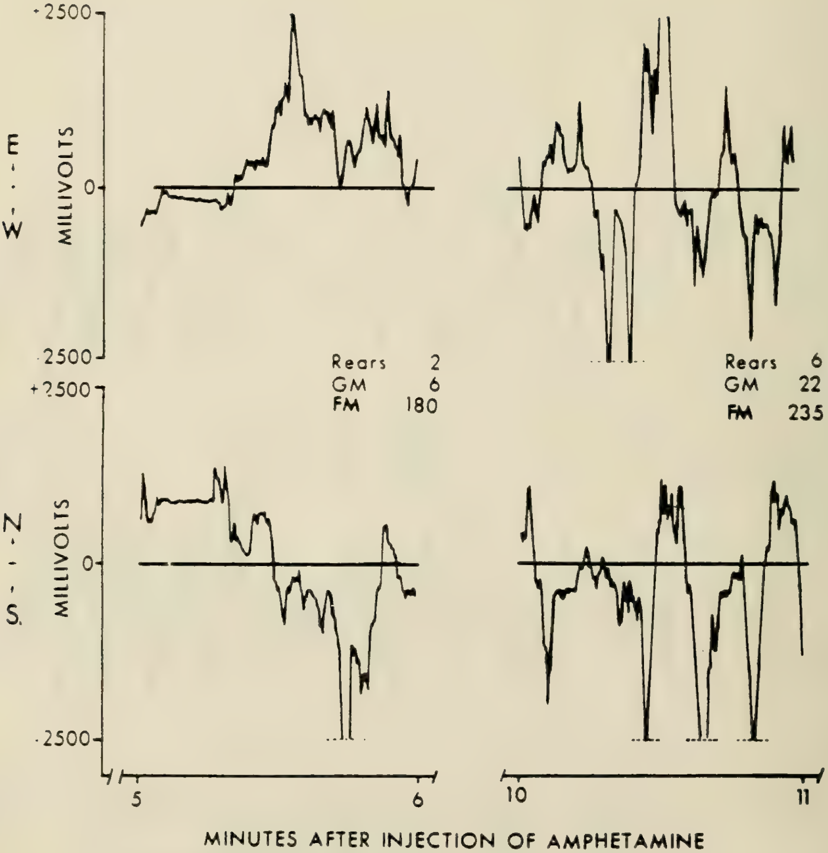
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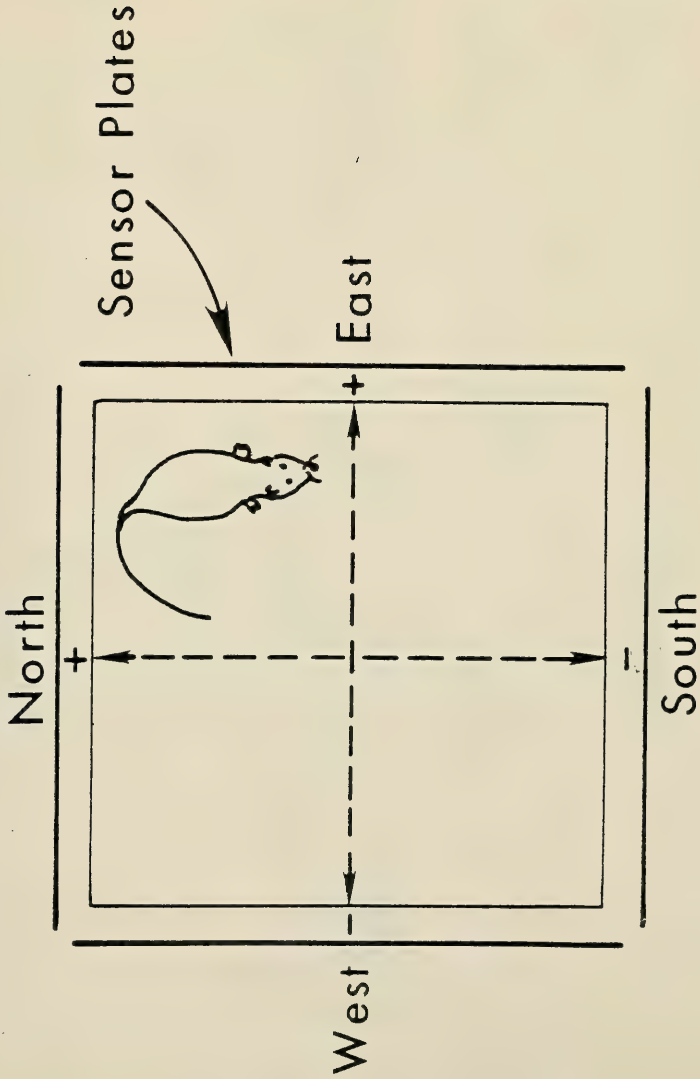
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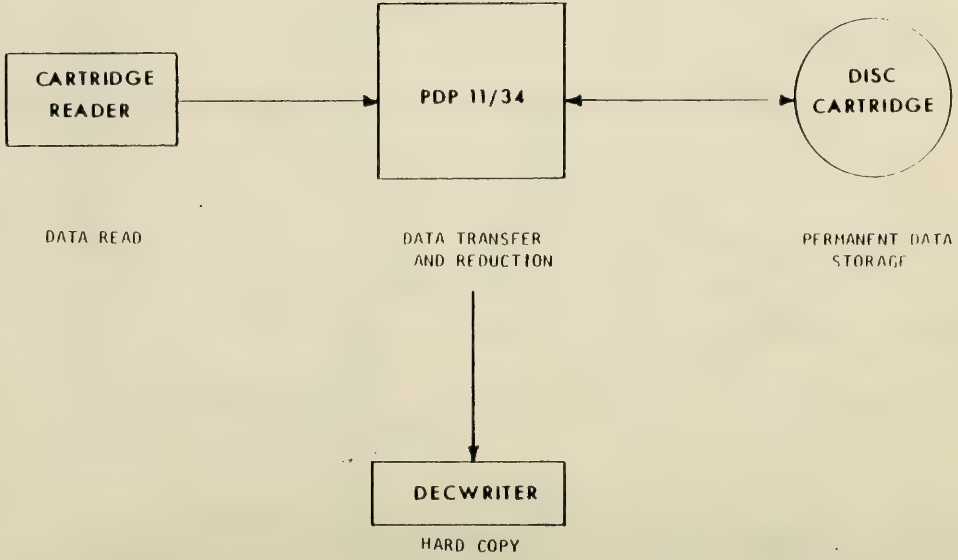

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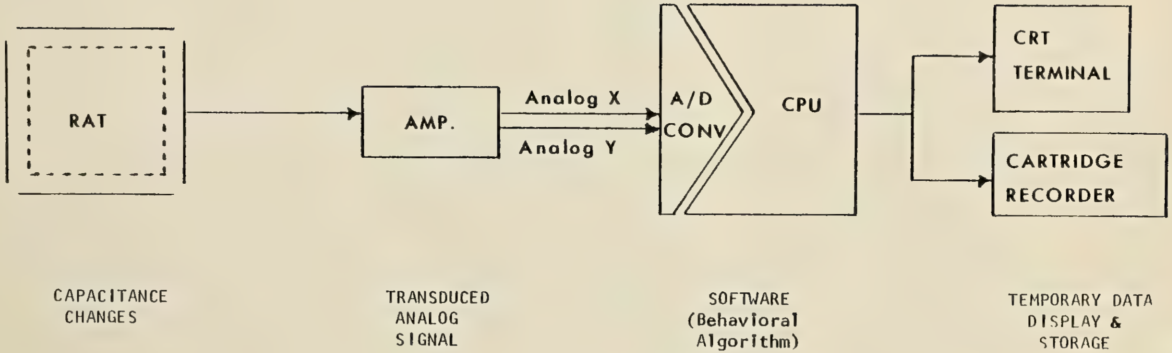

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01338-02 SMRC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies on Aging.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.J. Wyatt, Chief, Lab. of Clinical Psychopharmacology, J.C. Gillin, Deputy Chief, Laboratory of Clinical Psychopharmacology, SMRC; W.B. Mendelson, Staff Psychiatrist, SMRC; D. Shore, Staff Psychiatrist, SMRC; D.R. Weinberger, Staff Psychiatrist, SMRC; M.J. Perlow, Staff Neurologist, SMRC; W.J. Freed, Staff Fellow, SMRC; B. Phelps, Staff Fellow, SMRC; L.M. Neckers, Staff Fellow, SMRC; D.V. Jeste, Visiting Associate, SMRC; T.P. Bridge, Staff Psychiatrist, SMRC; OTHER: J. Brodie, NIA; B. Parker, NIAAA; S. Rapport, NIA; B.J. Hoffer, University of Colorado; L. Olsen, Karolinska Institute; A. Sager; Karolinska Institute; C. Gajdusek, NINCDS; H. Weingartner, DCBR; N. Sitaram, DCBR; M. Ebert, DCBR.

COOPERATING UNITS (if any) National Institute of Neurological Disease, National Institute of Aging, National Institute on Alcohol and Drug Abuse, Karolinska Institute, University of Colorado.

LAB/BRANCH
Laboratory of Clinical Psychopharmacology.

SECTION
Unit on Geriatric Psychiatry.

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 10	PROFESSIONAL: 6	OTHER: 4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To begin a broad based research program into the cognitive and emotional defects that occur with age.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To test existing and create new hypotheses relating to the social, psychological, cognitive and affective changes that occur with age.

Methods Employed:

The methods employed are directed at developing tools appropriate for studying older individuals.

Major Findings:

I. Drug Studies

A. Benzodiazepines

The benzodiazepines are the most commonly prescribed drugs in this country particularly in the elderly. Routine monitoring of benzodiazepine concentrations is desirable for many reasons. Patient dose requirements vary widely, which may be due to the large range of blood levels reported following the same dose. Because of benzodiazepine side effects, lethality when mixed with alcohol, and risk of physiological and psychological dependence appear dose related, patients should be maintained on the lowest effective therapeutic dose. Although threshold levels must be attained for therapeutic efficacy, excessive levels may cause clinical worsening. The principal method for measuring benzodiazepines, gas liquid chromatography, has not attained routine clinical use because of its technical complexities; also, development of a specific assay is required for each drug and its metabolites. We describe here an assay for benzodiazepines in saliva based on competition for diazepam receptor binding.

In this assay, saliva is used without any extraction or precipitation. Saliva is first centrifuged (10,000 g for 20 mins) to separate it into mucous sediment and serous supernatant. The serous supernatant is diluted by a factor of two with 50 mM Tris buffer pH 7.7 at 25°C and added directly to the assay. For the benzodiazepine radioreceptor assay, fresh rat frontal cortex was homogenized (Brinkman Polytron, setting 6 for 30 secs) in 50 volumes (w/v) 50 mM Tris buffer, pH 7.4 at 25°C. The homogenate was centrifuged twice at 50,000 g for 10 mins, with re-homogenization of the intermediate pellet in fresh buffer. The final pellet (which may be stored frozen) was resuspended in 15 volumes of freshly prepared 50 mM Tris buffer, pH 7.4 at 25°C. ^{3}H-diazepam (80 Ci mmol^{-1}, New England Nuclear) was diluted to 5 nM in fresh buffer. Polypropylene 12x75 mm incubation tubes received, in order, 100 λ diluted saliva (on benzodiazepine therapy or drug free), 50 λ 3H-ligand, 50 λ drug for standard curve or nitrazepam for blanks (final concentration 1 μM) or the drug solvent buffer, and tissue suspension to 0.5 ml total volume. Final concentration of ^{3}H-diazepam was 0.50 nM.

The tubes were incubated in triplicate at 0°C for 45 mins and rapidly filtered under vacuum through Whatman GF/B filters with two 5 ml rinses of ice cold 50 mM Tris buffer pH 7.4 at 25°C. ^{3}H-diazepam trapped on the filters was counted by liquid scintillation spectrometry after remaining overnight in scintillation vials containing Aquasol (New England Nuclear). A standard displacement curve for the drug under study was determined in the presence of equal volumes of control saliva with final concentrations of drug about one-third, three times, and the same as its IC50 value for inhibiting ^{3}H-diazepam binding under the conditions described. A log-probit plot was used to convert the displacement curve to a straight line, allowing percentage inhibition of ^{3}H-diazepam to be converted to molar drug concentration.

The absence of interference by saliva itself is apparent from experiments in which 100 λ of diluted saliva in a total assay volume of 0.5 ml reduced ^{3}H-diazepam binding by a mean ± SEM of only 1.60 ± .08%. Increasing volumes of saliva reduced binding linearly with about 45% inhibition occurring with 200 λ of diluted saliva. Saliva reduces specific and non-specific binding to the same extent, suggesting that saliva proteins bind ^{3}H-diazepam, making less available for interaction with brain membranes. The inhibition of binding by the saliva of several laboratory personnel was uniform, as was inhibition of binding by the same subjects' saliva collected on different days. Variability of inhibition of specific binding for repeated determinations of the same sample was less than 5%.

Benzodiazepines in saliva reduce the specific binding of ^{3}H-diazepam more than the small degree of inhibition caused by drug-free saliva, and the amount of inhibition of specific binding is proportional to the amount of benzodiazepine in saliva. The amount of benzodiazepine in a saliva sample can easily be determined using a standard curve of inhibition of ^{3}H-ligand binding by known amounts of the drug.

This method requires that benzodiazepines present in saliva will not inhibit non-specific (blank) binding of ^{3}H-ligand. In 14 saliva samples from patients on benzodiazepines, blank ^{3}H-diazepam binding was 231 ± 5 CPM, while non-specific binding in the presence of 12 control saliva samples was 205 ± 7 CPM. Thus, specific binding in the presence of patient saliva can be determined by subtracting non-specific binding values obtained with control saliva from total binding with patient saliva. The percentage inhibition of specific ^{3}H-diazepam binding (in the presence of control saliva) by the patient saliva (containing drug) is then calculated and compared to a standard displacement curve for determining actual benzodiazepine content.

Similar reductions in specific and non-specific binding of ^{3}H-diazepam by saliva indicates that saliva components may bind benzodiazepines making less available for membrane binding. In this assay, benzodiazepines bound to saliva components dissociate during the incubation so that total benzodiazepine levels are measured. To confirm this, 100 nM diazepam was pre-incubated for 10 mins at 37°C with control saliva to allow binding to saliva proteins to occur. The saliva with diazepam was then added to a standard binding assay. The time course and extent of reduction of binding was the same for diazepam pre-incubated with saliva and diazepam dissolved in buffer. The maximum percentage lowering of binding was equivalent to that produced by 100 nM diazepam added directly to the assay with no saliva present.

Recovery was examined as follows: four different benzodiazepines were preincubated with saliva for 10 mins at 37°C to provide for binding to saliva proteins and subsequently assayed for benzodiazepine levels. Diazepam (100 nM), chlordiazepoxide (1000 nM), nitrazepam (100 nM), and oxazepam (100 nM) were all fully recovered in the benzodiazepine radioreceptor assay with respective values of 97 ± 0.20 nM (n=4), 1000 ± .07 nM (n=4), 100 ± .46 nM (n=4), and 100 ± .13 nM (n=4).

The validity of the radioreceptor assay was assessed by comparing its results with saliva levels measured by another procedure. We measured saliva benzodiazepine levels in patients treated with oxazepam; saliva oxazepam levels were measured on the same samples by gas liquid chromatography with electron capture and by saliva radioreceptor assay. We found a good agreement between the different methods. Using 100 of two-fold diluted saliva, the lower limit of sensitivity (7% inhibition of specific binding) is 0.14 ng/ ml, 1.50 ng/ml, 0.28 ng/ml, and 2.87 ng/ml for diazepam, chlordiazepoxide, nitrazepam and oxazepam, respectively.

In summary, the simplicity of the benzodiazepine receptor assay for saliva, as well as the ease with which saliva samples (0.2 ml each) can be collected and stored, make this assay suitable for routine clinical use in large patient populations. Saliva can be collected at home and refrigerated for later assay. The assay is selective; chlorpromazine (10^{-5}M), haloperidol (10^{-5}M), fluphenazine (10^{-5}M), desmethylimipramine (10^{-5}M), pargyline (10^{-5}M), dl-propranolol (10^{-5}M), and levallorphan (10^{-5}M) incubated with saliva failed to inhibit ^{3}H-diazepam binding more than saliva itself, suggesting that this assay can be used in patients who are being treated with a variety of other drugs besides benzodiazepines. For patients receiving more than one benzodiazepine, the concentration of each cannot be determined separately. In such cases, inhibition of ^{3}H-diazepam binding can be converted to a "diazepam equivalent" which would produce the same degree of inhibition.

An advantage of this assay is that it would detect benzodiazepine metabolites that compete for 3H-diazepam binding and which may thus be clinically active. There is evidence that a major part of diazepam's clinical efficacy is due to its metabolites desmethyldiazepam and oxazepam. Both bind to the diazepam receptor and would thus be detected in this assay.

The radioreceptor assay for benozidazepine in saliva is simpler to perform than would be a radioreceptor assay for benzodiazepines in plasma or red blood cells. We have found a high degree of inhibition of 3H-diazepam specific and non-specific binding by both plasma and red blood cell lysates.

This would necessitate extra steps for further purification of plasma by deproteination and centrifugation. In the radioreceptor assay for benzodiazepines in saliva this is not necessary. Dilute serous saliva is added directly to the assay without further extraction or purification. Another possible advantage is that saliva benzodiazepine levels may more directly reflect intracellular drug levels, and thus may correlate more closely with clinical efficacy.

B. Neuroleptics

A sensitive radioreceptor assay for neuroleptic drugs in serum was used to examine the relationship between serum neuroleptic concentration and clinical response. Its validity was suggested by close correlation of serum concentration measured by both radioreceptor assay and radioimmunoassay; however, higher values were obtained with the radioreceptor assay, suggesting the possible presence of active haloperidol metabolites.

Repeated freezing and thawing of serum lowered serum neuroleptic concentration by 40-50%. However, brief exposure to rubber vacuum tube stoppers and storage of serum in glass did not affect serum neuroleptic concentration.

In a group of 24 haloperidol treated patients, daily dosage was correlated with haloperidol serum concentration. In another group of patients treated with several different neuroleptics, daily dosage was not correlated with serum concentration (in chlorpromazine equivalents) for the group as a whole; however, in subgroups of patients receiving the same neuroleptic, daily dosage was closely correlated with serum concentration. Ratios of serum neuroleptic concentration (in chlorpromazine equivalents) to daily dosage (in chlorpromazine clinical equivalents) differed for different neuroleptics. Serum and CSF neuroleptic concentration were significantly correlated as reported previously. Serum neuroleptic concentration and plasma prolactin were also closely correlated.

There was a significant negative correlation between age and neuroleptic serum concentration. Despite lower daily dosages, older patients had serum neuroleptic concentrations as high, and in many cases, higher than patients under 45 years of age.

In 10 patients at the onset of extrapyramidal reactions, neuroleptic serum levels were relatively low. Seven out of the 10 patients had serum neuroleptic concentrations below 100 ng/ml chlorpromazine equivalents. Also, despite similar neuroleptic dosage, patients treated conjointly with benztropine mesylate (2 mg p.o. b.i.d.) had significantly lower neuroleptic serum levels. Unlike patients with EPS, patients with tardive dyskinesia were found to have significantly higher serum neuroleptic concentrations than control patients matched for age, weight, drug, dosage, length of neuroleptic treatment, and diagnosis.

In 14 chronic schizophrenic patients at a fixed neuroleptic dosage for at least 14 days, anxiety/depression and thought disorder clusters of the Brief Psychiatric Rating Scale (BPRS) were negatively correlated with serum neuroleptic concentration. In the same group of patients, however, daily neuroleptic dosage was also negatively correlated with the same two symptom clusters. None of the other BPRS clusters, including anergy, activation, hostility, or global response was significantly correlated with either neuroleptic dosage or serum level. Both anxiety/depression and thought disorder ratings were significantly higher in patients with serum levels below 150 ng/ml (a threshold serum level found for appearance of detectable CSF neuroleptic.)

C. Flurazepam

The metabolic fate of flurazepam has been studied in animals and humans by using radioactive tracer techniques, spectrophotofluorometry and gas chromatographic techniques. Most of these studies described the pharmacokinetics of flurazepam in plasma or urine samples and indicate that N_1-desalkyl flurazepam is slowly biotransformed and accumulated during chronic therapy. Because the N_1-unsubstituted metabolite of this drug is pharmacologically active, it may be responsible for the unwanted toxic effects of flurazepam in the elderly following a 30 mg per night dose regime. The present experiments were designed to study the accumulation of lipophilic metabolites in the central nervous system of the cat after i.v. administration of 30 mg/kg of flurazepam. A gas chromatographic-mass spectrometric procedure is used in order to identify flurazepam and its metabolites in cerebrospinal fluid and plasma samples. A positive identification of benzodiazepines by mass spectrometry requires at least 50 to 100 ng per injection of sample into the Hewlett-Packard-5992 gas chromatographic-mass spectrometric system. Results confirm that the major metabolite of flurazepam in the blood was the N_1-desalkyl analogue, which accumulated significantly up to 24 hr after administration of a single dose of flurazepam. A positive identification of unchanged and N_1-desalkyl flurazepam in the cerebrospinal fluid was obtained 60 min after i.v. administration of the drug. There was an accumulation of this N_1-unsubstituted metabolite in the cerebrospinal fluid 24 hr after a single dose of flurazepam. The half life of flurazepam in plasma and cerebrospinal fluid appeared to be less than 2 hr. In summary, after a single i.v. administration of flurazepam, the unchanged analogue disappears quickly in plasma and cerebrospinal fluid while the N_1-desalkyl metabolite is slowly accumulated in blood and cerebrospinal fluid.

D. Memory

Studies conducted in the Unit on Sleep Studies in collaboration with Drs. Ebert and Weingartner at the Clinical Center suggest that normal volunteers learn more rapidly when treated with arecoline, physostigmine, or choline. Conversely, they learn less well when treated with scopolamine. These results indicate that learning and memory are fascilitated by the cholinergic system. Studies are currently underway, on the effects of arecoline and lecithin (a sympathetic precursor of acetylcholine) in patients with dementia.

E. Dementia Reduction of 5-Hydroxytryptophan

Markedly decreased levels of 5-hydroxytryptamine (5-HT), as assayed by high pressure liquid chromatography, have been found in brain tissue and blood of mice with advanced scrapie disease compared to control mice. Normal levels in brain were 340 ng 5-HT/g while less than 20 ng/g could be detected in scrapie affected mice. Normal levels in blood were 769 ng/ml versus 462 ng/ml in affected animals. In hamsters, blood levels in uninoculated age matched animals were 258 ng/ml, and in animals sham inoculated with normal brain they were 319 ng/ml, whereas in scrapie affected animals they were 118 ng/ml.

Whether this greater than ten-fold decrease in 5-HT levels in brain and two-fold decrease in blood indicates a primary loss in synthetic ability, an enhanced catabolism, or a new binding activity for 5-HT secondary to the pathological changes is as yet unknown. Analysis of 5-HT can, however, be used to follow the course of infection in laboratory animals and the natural diseases.

F. Aluminum

Several investigators have noted that patients with senile dementia have high aluminum concentrations in the cerebral cortex and other tissues and suggest that aluminum may be a causative agent in certain dementias of old age. We have been taking blood samples from patients with senile dementia and age-matched controls. Serum samples were sent to U. of Va. Med. Coll. for aluminum assays and to San Pedro, Calif. for parathyroid hormone (PTH) determinations (since PTH causes aluminum accumulation in cerebral cortex).

Our failure to find significant differences in serum aluminum or circulating PTH in senile dementia patients is consistent with other studies. We find no indication that the reported elevations of cerebral cortex aluminum in senile dementia patients are the result of a generalized tissue overload as may occur in patients on dialysis.

While abnormal accumulation of aluminum in senile dementia (or Alzheimer's disease) patients' brains may occur, the reasons for and significance of this accumulation remain unclear. Our findings of normal serum and CSF aluminum in senile dementia patients indicates that nuclear aluminum accumulation is not likely the result of a generalized overload of this metal in biological fluids. Further study of the interaction between aluminum and CNS nuclear chromatin may show why the aluminum attaches precedes or follow neurologic deterioration, and how cortical aluminum accumulation might be prevented or reversed.

G. Paraphrenia

American psychiatry has traditionally adopted the view that late life paranoid states are rare and that paraphrenia (late onset paranoid schizophrenia) should not be recognized as a diagnostic entity. A review of world literature strongly suggests that this group of patients should not be discussed so casually.

Multiple studies outside the United States concur that late life paranoid states or paraphrenia represent about 10% of all first admission psychiatric patients over the age of 60. Furthermore, there is evidence that this disorder has premorbid characteristics that distinguish it and that there is a genetic determinant to this particular disease. Diagnostic reliability for paraphrenia can be demonstrated. In fact, cross national studies between the United States and the United Kingdom indicate that United States psychiatrists most often misdiagnose hostile, belligerent, paranoid patients as organic rather than functional. This assumes great importance when one considers that nearly universal treatment response to phenothiazines among these patients that is reported in the European literature. Finally this group of patients is of interest for its research potential. Both symptomatically and genetically this group appeared to be intermediate between younger chronic schizophrenics and the population at large. As such, they can provide opportunity to learn, for example, about the timing of onset of psychosis, the effects of aging upon psychoses, or the relationship of paranoid psychoses- personality integration. Most importantly, evidence would suggest that this group deserves diagnostic recognition in order to provide them access to treatment which has proven so efficacious for them.

H. Platelet Monoamine Oxidase

Observations have been made previously that platelet, plasma and brain tissue monoamine oxidase (MAO) activity is elevated in older individuals relative to younger subjects. This evident increase in enzyme activity with advancing age is in contrast to the other catecholamine enzymes. In addition, to advancing age, certain pathologic states have been associated with elevated platelet MAO activity including depressive disorders, senile dementia, and glaucoma.

Population studies of other catecholamine enzymes have demonstrated that very low enzyme activity is under genetic control, probably inherited as an autosomal recessive trait. Susceptability to heat inactivation (thermolability) believed to be one of the most sensitive indicators of altered enzyme amino acid sequence, is under the control of the structural gene for the enzyme. Very low thermolability, which correlates with low enzyme activity in the general population samples is also evidently inherited as an autosomal recessive. Within families where both traits are present, however, thermolability and low enzyme activity sort independently for these other catecholamine enzymes.

Data are reported from a general population study (N=237) of platelet MAO activity in a demographically diverse sample without evidence of psychiatric illness, often associated with altered platelet MAO activity. Within the entire sample, age correlates positively with enzyme activity (r=.49). No such relationship is present, however, in those subjects aged 18-50 or those aged 51-79. The decade 50-59 shows the bulk of the increment in enzyme activity. In older individuals in this sample (age 50) there is a phenomenon of increased thermolability relative to younger subjects. This finding in the older subjects is opposite to the usual association of low enzyme activity and thermolability.

In separate populations of older schizophrenic patients (N=27), nursing home residents (N=15) without evidence of functional psychopathology, and elderly residing in the community (N=43), activity of two catecholamine enzymes, monoamine oxidase (MAO) and dopamine-beta-hydroxylase (DBH) were measured in platelets and plasma respectively. Separate assessments of functional psychopathology by the Research Diagnostic Criteria and cognitive function by the Folstein Mini-Mental State Exam were made by board certified psychiatrists blind to individual subjects' enzyme activity. Data are presented demonstrating age dependent relationships of enzyme activity and congnitive function. Within these three groups, evidence is found for distinct patterns of enzyme activity for both functional psychopathology and cognitive function.

I. Lymphocyte MAO

In our studies of lymphocyte MAO, we have examined its presence in the two major subpopulations of lymphocytes, thymus-derived (or T lymphocytes) and bursal equivalents (or B lymphocytes). MAO is not evenly distributed between these two subpopulations, but occurs in much greater frequency in the B lymphocyte. In controls, 79% of total lympocyte MAO occurs in B cells, 9% in T cells and the remainder in non-resetting, non-adherent cells. In our schizophrenic patients, only 64% of the total activity occurs in B cells, 17% in T cells and the remainder in non-resetting, non-adherent cells. This suggests a different distribution of the enzyme in healthy and schizophrenic individuals. We are currently investigating this phenomenon in aged individuals. We are currently investigating this phenomenon in aged individuals.

J. Brain Grafts

Many neurological disorders are associated with degeneration of discrete populations of neuronal elements. Parkinson's Disease, for example, manifested primarily by abnormalities of movement and posture, is characterized by neuronal loss and gliosis in the dopamine-containing zona compacta of the substantia nigra. Neurochemically, the syndrome is characterized by decreased concentrations of dopamine (DA) and its preincipal metabolite, homovanillic acid (HVA) in the neo- and paleo-striatum (caudate, putamen, globus pallidus) and substantia nigra.

Current therapeutic approaches to Parkinson's Disease involve peripheral administration of L-DOPA, a precursor for dopamine, and dopamine-like agents. In practice, despite some dramatic improvements, such therapeutic regimens are frequently not completely effective, or associated with severe untoward side effects. Many of these difficulties may result from: (i) use of a precursor rather than the transmitter substance itself; (ii) generalized distribution of the drugs in brain rather than focal release from terminals in discrete areas; and (iii) absence of the physiological mechanisms which normally regulate transmitter release from dopaminergic terminals. We have attempted to circumvent these problems by using central nervous system grafts as a source of dopamine in an animal model of substantia nigra degeneration. We now report that fetal rat substantia nigra neurons can be homologously transplanted in juxtaposition to dopamine-denervated host adult caudate. The grafts proliferate extensively *in situ* and establish a behaviorally appropriate functional input to the denervated caudate.

As an experimental model, we chose the rat with unilateral destruction of the substantia nigra DA system by 6-hydroxydopamine 96-OHDA). Within one week following an unilateral injection of 6-OHDA into the substantia nigra, DA-containing cells in that site, and their axonal terminals in the corpus striatum degenerate; DA concentrations in the corpus striatum are greatly reduced. To compensate for the loss of DA, it is postulated that striatal neurons develop increased sensitivity to DA. This "supersensitivity" is manifested as: increased numbers of DA receptor sites and increased DA-stimulated adenylate cyclase activity, a decrease in the threshold of caudate neurons to the inhibitory effects of iontophoretically applied DA agonists, and a pronounced rotation contralateral to the lesion, after systemic administration of the DA receptor agonist, apomorphine. If a brain graft developed a DA input to the denervated caudate nucleus, we postulated that the supersensitivity, manifested as apomorphine-induced rotation, would be reduced.

Unilateral destruction of DA cell bodies in male Sprague-Dawley rats (150-160 gm) was accomplished using injection of 6-OHDA into the right substantia nigra and ascending DA axon bundle. After 2-4 months, the rats were placed in a rotometer and given a subcutaneous injection of apomorphine hydrochloride (0.1 or 0.25 mg/kg body weight). The number of clockwise and counterclockwise turns was recorded separately for each animal at 5-minute intervals for 40 minutes. Testing was repeated twice a week for 3-4 weeks.

Rats that had stable patterns of rotation were subjected to grafting operations. Pieces of fetal ventral mesencephalon containing the substantia nigra (SN) or adult sciatic nerve were dissected out and injected into the lateral cerebral ventricle ipsilateral to the 6-OHDA lesion. Baseline turning rates were not statistically different for the animals in SN and sciatic nerve implant groups prior to transplantation ($p < 0.50$, F test).

Four weeks after grafting, animals were again tested for rotation in response to the same dose of apomorphine (twice a week for 3-4 weeks). By and large, most animals did not rotate as much after grafting as before, but the reduction in turning was significantly greater for animals grafted with SN tissue than for animals with sciatic nerve implants. Data were analyzed statistically in terms of the ratios of the mean turning rate prior to transplantation, for each of the eight 5-minute segments of the 40-minute testing session. A two-way analysis of variance (groups x measures) revealed a significant main effect of the type of graft ($F=5.36$, $dF=1$, $p < 0.03$). The main effect of measures (comparing 5-minute segments) was not significant.

The caudate nucleus and periventricular tissue of 12 of the 29 animals studied were examined using Falck-Hillarp fluorescence histochemistry and a double-blind protocol. Five of these animals had SN grafts and had at least a 70% reduction in their rotatory response to apomorphine (R animals). Four of 12 had SN grafts but less than 10% reduction in rotatory response to apomorphine (NR animals). The remaining three had sciatic nerve grafts (controls).

Although the sciatic nerve fibers degenerated after grafting, the histochemical studies revealed excellent survival and growth of the ventral mesencephalic SN grafts. Of 30 monoamine-containing implants examined in 9 animals, all but one showed large numbers of catecholamine and serotonin containing neurons with a morphology typical of adult substantia nigra and raphe, respectively. The catecholamine and serotonin containing cell bodies formed one or more dense clusters in the graft which were clearly separate. Monoamine containing fibers proliferated extensively within the grafts and invaded the adjacent caudate in all nine animals with SN implants. The catecholamine fibers that grew into the denervated caudate formed a network only in the part of the caudate adjacent to the graft. A similar ingrowth was seen into the lateral septum. However, no monoamine containing fibers invaded the corpus callosum, even at points of attachment of the graft. Monoamine containing fibers also formed dense sheets of fibers within the ependyma of the lateral ventricle. For the most part, anteriorly placed grafts formed well-demarcated structures in the lateral ventricle opposed to or fused with the medial surface of the caudate and lateral surface of the septum. Some deeper grafts lay interstitially, fused with the host brain tissue. Transplants that were placed more posteriorly in the lateral ventricle were usually found adjacent to or fused with the fornix and dorsal alveus of the hippocampus. There was little or no evidence of any scaring or pathological disturbances of the host striatum.

It was of great interest to compare the histochemical properties of grafts in animals that showed reduced turning after grafting with those that did not. Both groups had approximately equal mean numbers of catecholamine-containing cell bodies per animal and proliferating fibers in the graft. Moreover, the ingrowth of fibers into the adjacent caudate was approximately equal in both groups. The major difference in the two groups was in the degree of caudate denervation produced by the 6-OHDA injection. The degree of initial 6-OHDA denervation could be estimated by histochemical examination of the host's caudate, remote from graft, and by counting the remaining dopamine-containing cells in the host's substantia nigra. All R animals were virtually totally denervated. Every NR animal had some residual endogenous DA-containing cell bodies in the nigra and weak to very weak diffuse endogenous DA fluorescence in large areas of the caudate. A second difference was in the location of the graft. NR animals tended to have a more posterior graft localization within the ventricle, while R animals had a more anterior placement.

From the above studies, we conclude the following: (1) The grafts of fetal SN to the lateral ventricle adjacent to the caudate nucleus of the rats survived and proliferated. All but one of the grafts examined survived without rejection for at least two months; (2) grafting of the fetal SN reversed, to some degree, the behavioral changes associated with destruction of the host nigro-striatal system; (3) this recovery was associated with the growth of DA-containing cells and fibers in the grafts and into host brain tissue. However, from the data reported here, the functional recovery cannot be ascribed strictly to re-innervation of the caudate; release of DA and subsequent diffusion from the grafts into the caudate is another possibility (4) Incomplete destruction of the endogenous DA input, although sufficient to induce "behavioral supersensitivity", may antagonize reversal of the supersensitivity by a subsequent SN graft. (5) The small reduction in turning seen in control animals may result from a disturbance in caudate function secondary to grafting procedure, although histological examination did not reveal evidence of caudate injury.

Although numerous studies have demonstrated (i) the regeneration of mammalian nervous tissue following injury, (ii) that various parts of the mammalian central nervous system can be grafted to peripheral tissue, and (iii) that non-nervous tissue can be grafted to the brain, there have been few studies demonstrating that central nervous system tissue could be grafted to and survive in the brain or spinal cord of nother animal. In some studies where the grafted nervous tissue has been placed into host brain areas, fiber proliferation and synapse formation have been demonstrated. This is, to our knowledge, the first demonstration, however, that the grafting of mammalian brain tissue from one animal to another elicits an alteration in the behavior of the recipient animal consistent with the normal function of the grafted tissue.

Parkinson's Disease has a wide variety of signs and symptoms: not all of these are reliably improved by current therapy. Our data show that catecholamine-containing tissue, implanted in proximity to the caudate survives, proliferates and can produce improvements in lesion-induced motor abnormalities. While clearly much additional research is required to establish the safety and long term stability of grafts, there is a possibility that such a local catecholamine source could offer the patient with Parkinson's Disease a more physiological treatment than is presently available. In this context, it is important to explore autografts and/or homografts of peripheral catecholamine-containing tissues, such as adrenal medulla. If further studies can establish the efficacy and safety of this approach for therapy, it may eventually be possible to employ techniques similar to those that we have used here in the treatment of other neurological disorders where the loss of nerve cells is reasonably well circumscribed.

K. Brain Neurotransmitter Receptors

We demonstrated high affinity stereospecific binding of the dopaminergic ligand 3H-spiroperidol to rat renal membranes. The binding was saturable, regionally distributed, and showed the same specificity as the caudate 3H-spiroperidol site for displacement by dopaminergic antagonists. Since there is an association between hypertension and abnormalities of renal blood flow, which appears to be regulated in part by dopamine, 3H-spiroperidol binding was measured in kidneys of spontaneously hypertensive rats. In two independent experiments renal cortical 3H-spiroperidol stereospecific binding was increased in spontaneously hypertensive rats, raising the possibility that some forms of hypertension may result from abnormalities of renal dopamine receptors.

Guanine nucleotides play a role in the activation of adenylate cyclase. Although the mechanism by which GTP apparently mediates adenylate cyclase activation is not known, it is thought to involve a guanine nucleotide binding protein associated with both the cell surface hormone receptor and adenylate cyclase. We therefore studied the binding of 3H-GTP to rat brain homogenates. 3H-GTP binding was saturable; Scatchard analysis revealed a higher affinity lower capacity binding site (Kd=2.0 nM, Bmax=164 pmoles/gm tissue) and a lower affinity higher capacity site (Kd=13 nM, Bmax=690 pmoles/gm tissue). Association was rapid, reaching equilibrium within five minutes; by fifteen minutes of incubation 30-40% of 3H-GTP binding dissociated spontaneously. Scatchard analysis at this incubation time revealed only the higher affinity component of binding. By 45 minutes following addition of 100 micromolar unlabelled GTP at equilibrium 80-90% of 3H-GTP binding had dissociated. GTP was ten and one hundred times more potent in displacing 3H-GTP binding than GDP and CTP, respectively; guanosine and guanine failed to inhibit 3H-GTP binding at concentrations less than 1 mM. ATP was completely inactive in displacing 3H-GTP. 3H-GTP binding was reduced by over 90% following exposure of brain homogenate to 53 degrees Cent. for 15 minutes. Binding of 3H-GTP was relatively similar in all brain areas studied (caudate, cerebral cortex, hippocampus, cerebellum, hypothalamus, and medulla-pons). It is hoped that direct binding techniques will prove useful in studying interactions among agonists, cell surface receptors and adenylate cyclase.

L. Tardive Dyskinesia

Tardive Dyskinesia (TD) is considered a major limitation to treatment with neuroleptics. There are, however, controversies regarding its incidence, etiopathology, course, prevention and treatment.

Twenty-one patients over the age of 50, who were admitted to New York (Westchester division) during a one-year period, and who had tardive dyskinesia were followed for a mean of 13 months. Neuroleptics and antidepressants were discontinued for at least three months. Dyskinetic symptoms abated in 12 patients, and persisted in 9. Discriminant function analysis showed that the persistent and reversible dyskinesia groups could be clearly separated and that the one best discriminator was the number of drug-free intervals. The persistent dyskinesia group had significantly longer neuroleptic treatment and greater number of drug interruptions than the reversible dyskinesia group. Our findings raise doubts about the commonly recommended practice of frequently interrupting long-term drug treatment, at least in patients who are otherwise predisposed to develop tardive dyskinesia.

Tardive dyskinesia has been suggested that TD may be associated with structural damage in striatal area. Because of its reportedly increasing incidence, and irreversibility in some cases, there is concern about medicolegal aspects of TD. Yet, the few published studies on postmortem and antemortem neuropathologic findings in TD have yielded inconclusive and conflicting data. We wished to compare computerized tomography (CT) scans of patients with severe and long-lasting TD with those of three groups of patients: (A) Non-TD psychiatric controls matched for age, sex, race, primary psychiatric diagnosis, and neuroleptic and other treatment history. Differences between CT scans of this group and TD group might suggest some "risk factors" for development of TD. (B) Huntington's chorea patients. A number of clinical similarities between TD and Huntington's chorea have led to a suggestion that these two movement disorders may have similar underlying neuropathology (caudate atrophy). (C) Age-matched normal controls as reported in literature.

We screened inpatients from two female geriatric units of a psychiatric hospital. A diagnosis of TD was made by two psychiatrists independently using strict criteria (Jeste *et al.*, Arch. Gen. Psychiat., in press). Severity of TD was rated on standardized rating scales. Thorough psychiatric, neurologic, dental and other physical evaluations were done, and detailed history of past treatments obtained. CT scans were performed with EMI scanner on 12 patients with severe TD (duration many months to several years), and 12 matched psychiatric controls. Patients' mean age was 68.6 and mean duration of hospitalization 35.5 years. Most patients were chronic schizophrenics. CT scans (8 to 12 cuts per subject) were read "blind" by two persons for signs of cortical atrophy (Huckman's criteria), ventricular enlargement (Hahn and Huckman's criteria), caudate atrophy (bifrontal: bicaudate ratio), cerebellar atrophy (Allen's criteria) and focal abnormalities. These scans were also compared to 42 scans of patients with Huntington's chorea done earlier.

No specific differences were found between TD patients' scans and those of matched psychiatric controls. TD was not associated with caudate atrophy, unlike Huntington's chorea. Interestingly, 85% of our patients over 60, with long-term neuroleptic treatment (both in TD and non-TD groups) showed cortical atrophy and/or ventricular enlargement. By contrast, Huckman et al. (1975) found normal CT scans in 40% non-demented persons over 60. Further work is needed before interpreting this.

The above findings, which are a part of an ongoing study, indicate a need for a different look at neuropathology of TD.

We are presently conducting a series of animal experiments to test the effect of drug interruptions on a model of tardive dyskinesia. We studied two aspects of this condition - prevention of and susceptibility to TD in an animal model. The model involved demonstration of behavioral supersensitivity to amphetamine following long-term oral administration of haloperidol to rats. The results indicate that (I) intermittent administration of haloperidol does not retard development of supersensitivity and (II) initial low responders to amphetamine develop greater percentage increase in activity counts to response to amphetamine following chronic haloperidol treatment than do initial high responders. These findings suggest two clinically important and testable hypotheses: (I) intermittent neuroleptic treatment may not prevent TD, and (II) initial amphetamine response may predict susceptibility to TD.

The most popular theory of the pathophysiology of neuroleptic-induced TD attributes the movement disorder to postsynaptic dopamine receptor supersensitivity in the nigrostriatal system. This hypothesis is based on circumstantial evidence. A consideration of the available clinical and animal data suggests the following: (1) There is nigrostriatal catecholaminergic overactivity in TD. Such overactivity could result from presynaptic and/or postsynaptic disturbances. (2) Postsynaptic dopamine receptor supersensitivity is probably a normal consequence of neuroleptic administration and is not sufficient to explain why TD develops in only a proportion of patients on long-term neuroleptic treatment. Postsynaptic supersensitivity may be responsible for withdrawal dyskinesias, but clinical studies do not support the supersensitivity theory in most patients with TD. (3) Presynaptic dopaminergic and/or noradrenergic hyperactivity may be the crucial factor necessary for the induction of TD, or at least of most of its subtypes.

Publications:

1. Rosenblatt, J.E., Bridge, T.P. and Wyatt, R.J.: A novel method for measuring benzodiazepines in saliva. Communications in Psychopharmaology, 3:49-54, 1979.

2. Perlow, M.J., Freed, W.J., Hoffer, B.J., Seiger, A., Olson, L., and Wyatt, R.J.: Brain grafts reduce motor abnormalities produced by destruction of the nigro-striatal dopamine system: Behavioral and histochemical evidence. Science 204:643-647, 1979.

3. Bridge, T.P., Cannon, H.E., and Wyatt, R.J.: Burned out schizophrenia: Evidence for age effect on schizophrenic symptomatology. Journal of Gerontology 33: 835-839, 1978.

4. Jeste, D.V., Gillin, J.C. and Wyatt, R.J.: Serendipity in biological psychiatry--A myth? Archives of General Psychiatry, 36: 1173-1180, 1979.

5. Jeste, D.V. and Wyatt, R.J.: Tardive dyskinesia. The syndrome. Psychiatric Annals, 10: 16-25, 1980.

6. Jeste, D.V. and Wyatt, R.J.: In search of treatment for tardive dyskinesia: A review of the literature. Schizophrenia Bulletin 5: 251-293, 1979.

7. Jeste, D.V., Wagner, R.L., Weinberger, D.R., Rieth, K.G., and Wyatt, R.J.: Evaluation of CT scans in tardive dyskinesia. American Journal of Psychiatry 137: 247-248, 1980.

8. Jeste, D.V. and Wyatt, R.J.: Tardive dyskinesia: A review of treatment possibilities and their effectiveness. Psychiatric Annals 10: 26-38, 1980.

9. Jeste, D.V. and Wyatt, R.J.: Prevention and management of tardive dyskinesia: Guidelines for the use of neuroleptics in clinical practice. Psychiatric Annals 10: 39-52, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01339-05 SMRC

PERIOD COVERED
October 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Eye Tracking in Schizophrenic Patients.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas D. Reynolds	Chief, Section on Psychophysiology of Schizophrenia	SMRC
OTHER:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eye tracking of schizophrenic and normal controls is being explored. Pilot study suggests simple attentional deficits, correctable by making stimulus situation more interesting. Schizophrenic subjects in our study show minimal deficit.

Project Description:

Objectives:

To attempt replication of reported finding of defective eye-tracking in schizophrenia, and correlate with CAT scan results.

Methods Employed:

Electrooculogram is recorded while subject follows a pendulum movement. Various stimuli are affixed to pendulum bob.

Major Findings:

Over 40 patients have been studied. Eye-tracking in schizophrenia most markedly improved by affixing picture of a face on the bob. Similar findings have been reported, but our data suggest schizophrenia tracking more closely approximate normal tracking than hitherto reported.

Significance to Bio-Medical Research and the Program of the Institute:

Utility of eye-tracking as a genetic marker rendered doubtful. If correlated with CAT scan data (ready for analysis) may have more specific significance.

Proposed Course:

We expect to continue to use apparatus to correlate with other measures of cognitive function and anatomical deviances.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01492-08 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Properties of monoamine oxidases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. H. Neff	Chief	SMRP-B	NIMH
Other:	R. J. Wyatt	Chief	SMRC	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to survey the types and activities of monoamine oxidase (E.C. 1.4.3.4) in autopsy material from individuals with various psychiatric disorders.

PHS-6040
(Rev. 10-76)

Proposed Course:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01500-08 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Indolealkylamines and neuronal function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Z. Lackovic	Visiting Associate	SMRP	NIMH
Other:	M. Parenti	Visiting Associate	SMRP	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our objective is to develop a simple method for the analysis of indolealkylamines and related substances and to use this method to determine whether serotonin might play a role in peripheral nerve function.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective is to determine whether serotonin might play a role in the processing of information in the peripheral nervous system. The project was divided into two parts: 1) identification and quantitation of serotonin and related substances; and 2) distribution of serotonin in peripheral tissues.

A new high pressure liquid chromatography (HPLC) method was developed for the measurement of serotonin, 5-hydroxytryptophan and 5-hydroxyindoleacetic acid in animal and human autopsy samples. In initial studies, we identified serotonin and 5-hydroxyindoleacetic acid in human spinal nerve roots by gas chromatography mass spectrometry (GC-MS). We developed a HPLC technique with electrochemical detection to overcome nonlinear conversion of serotonin to a derivative suitable for GC-MS analysis. The new method requires minimal sample preparation and is sensitive to the presence of femtomole quantities of 5-hydroxyindoles.

Preliminary studies with human and animal tissue revealed that serotonin and 5-hydroxyindoleacetic acid are present in dorsal and ventral nerve roots. Moreover, there appears to be a differential distribution of serotonin and its metabolites in blood vessels. For example, serotonin is generally found in higher concentrations in nerves than in arteries. Within the human iliac artery serotonin is mostly concentrated in the tunica media, the smooth muscle containing region of the vessel. These observations suggest that serotonin may play a role in peripheral nerve function. Hopefully, our studies will provide a better understanding of the physiological role of peripheral serotonin and how drugs might interact with the system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01503-06 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Pharmacology studies of acetylcholine turnover: Control of cholinergic pathways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Brunello	Visiting Associate	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine agonists, muscimol and β-endorphin reduce the turnover rate of acetylcholine in hippocampus by an action in septum. Intraseptally injected bicuculline is able to reverse these effects supporting the presence of septal GABA-ergic interneurons. Intraseptal (lateral) kainate reduces septal glutamic acid decarboxylase, but not hippocampal choline acetyltransferase. In animals under pentobarbital anaesthesia the turnover rate of acetylcholine is markedly reduced in the hippocampus and in the cortex. The behavioral effect of pentobarbital is partially antagonized by the intraseptal administration of bicuculline, kainic acid and thyrotropin releasing hormone and the decreased ACh turnover is specifically reversed in the ipsilateral hippocampus but not in the contralateral hippocampus or in either side of the frontal cortex.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of this study was to improve our knowledge of the neurochemistry of the local circuitry regulating the cholinergic pathways.

In an effort to gain a better understanding of the serotonergic regulation of acetylcholine neuronal systems, we evaluated the effects of various serotonin agonists, including LSD on acetylcholine turnover in the frontal cortex, striatum, and hippocampus. The serotonin agonists employed were quipazine, MK 212, i-m-chlorophenylpiperazine and LSD. Of these compounds quipazine appears to be unique in that it elicited a significant increase in striatal acetylcholine turnover. MK 212 increased the percent of label incorporated into both choline and acetylcholine in the striatum and hippocampus.

The methods for determining the concentration and turnover rate of acetylcholine were described in Project MH 01509-08 SMRP-1978.

Injection of the GABA agonist muscimol (0.88 nmole) into the septum decreases the turnover rate of acetylcholine in the hippocampus; the GABA antagonist, bicuculline, has no effect. The lack of action by bicuculline may suggest that the GABA interneurons do not exert a tonic influence on the firing of hippocampal cholinergic neurons. However, bicuculline blocks the inhibition elicited by muscimol, β-endorphin or apomorphine on the hippocampal cholinergic system. This would suggest that both dopamine and β-endorphin exert their action on acetylcholine turnover in hippocampus via the GABAergic interneurons in the septum.

Chronic kainic acid (1 μg, 15 d) injected directly into the medial septum fails to alter hippocampal acetylcholine turnover rate, whereas kainic acid injected in the lateral septum causes a decrease in the ipsilateral septal glutamic acid decarboxylase without any effect on hippocampal choline acetyltransferase. Since GABAergic interneurons of the lateral septum can be destroyed with no change to the cholinergic perikaria of the medial septum, we can answer many questions concerning the control of the cholinergic septal hippocampal pathway.

Another series of experiments has been performed in animals under pentobarbital anaesthesia. Under this condition the turnover rate of acetylcholine in the hippocampus as well as in both frontal and parietal cortex is markedly reduced. A reversal of the narcotic action of pentobarbital has been detected in animals injected in the lateral septum with bicuculline, with kainic acid or with thyrotropin releasing hormone. In the same animals the turnover rate of hippocampal acetylcholine is brought back to the value of unanaesthetized animals indicating that blockade of GABA receptors, stimulation of glutamate receptors and the interaction by thyrotropin releasing hormone with its own receptors (densely present in the lateral septum) may have the same regulatory effect on the cholinergic pathway.

To further characterize the neurochemical substrate underlying this antagonism of pentobarbital narcosis, it would be interesting to measure GABA turnover in the septum of pentobarbital-treated animals and to check whether each compound is acting through the same GABAergic interneurons.

Publications:

Cheney, D. L., Robinson, S. E., Malthe-Sørenssen, D., Wood, P. L., and Costa, E.: Regulation of hippocampal cholinergic neurons by dopaminergic synapses in septum. In Usdin, E., Kopin, J. S., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 1077-1079.

Robinson, S. E., Cheney, D. L., and Costa, E.: The regulation of hippocampal cholinergic neurons by noradrenergic mechanisms. In Usdin, E., Kopin, J. S., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 1062-1064.

Robinson, S. E., Cheney, D. L., and Costa, E.: Regulation of septal-hippocampal cholinergic neurons by catecholamines. Cholinergic Mechanisms. New York, Raven Press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01505-07 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Acetylcholine and γ-aminobutyric acid turnover rates: Anesthetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Cheney	Chief	SMRP-M	NIMH
Other:	A. V. Revuelta	Visiting Associate	SMRP	NIMH

COOPERATING UNITS (if any)
Dr. Edward Brunner, Dept. of Anesthesiology, Northwestern University
Chicago, Illinois

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although the anesthetic gases, halothane and enflurane, reduce the percent incorporation of ^{13}C-label into GABA in globus pallidus, caudate, accumbens and substantia nigra, the turnover rate of GABA was significantly reduced by both anesthetics only in accumbens and by enflurane in septum. The reduced turnover rate of GABA in septum following enflurane administration may be related to the excitatory activity produced by this compound.

Project Description:

Our objective was to study the mechanisms that regulate the function of cholinergic and γ-aminobutyric acid (GABA)-ergic neurons in the central nervous system following administration of gaseous anesthetics.

Glutamate and GABA concentrations were measured mass spectrometrically in various nuclei following infusion of ^{13}C-glucose (75 µmol/kg/min) for 10 min. The fractional rate constants for GABA efflux were determined from the ratios between the 5 percent of label incorporated in glutamate and GABA. By multiplying the fractional rate constant by the steady-state concentration, the turnover rate of GABA can be estimated. Gaseous halothane (1%; 40 min) and enflurane (3%; 40 min) were administered and the rats were killed by focussed microwave irradiation.

In preliminary experiments both halothane and enflurane reduced the percent incorporation of ^{13}-label into GABA in globus pallidus, caudate, accumbens and substantia nigra. In septum enflurane, but not halothane, reduced the percent incorporation of ^{13}C label into GABA. Only in globus pallidus was there any alterations in precursor/product concentration. In this nucleus halothane elevated the GABA content by 40%. The turnover rate of GABA was significantly reduced by both anesthetics only in accumbens and by enflurane in septum. While halothane is a pure central nervous system depressant devoid of excitatory actions, enflurane has been shown to induce cortical hypersynchronous seizure-like activity that originates in the limbic system. Thus the reduced turnover rate in septum following enflurane may be related to the excitatory activity produced by this compound. Further experiments will be necessary to substantiate these results.

Clarification of the influences exerted by various anesthetic agents on cholinergic and GABAergic mechanisms may help in understanding the role of GABA and acetylcholine in consciousness.

Publications:

Ngai, S. H., Shirasawa, R., and Cheney, D. L.: Changes in motor activity and acetylcholine turnover in brain regions of rats injected with lidocaine and cocaine. Anesthesiology 51: 230-234, 1979.

Cheney, D. L., and Ngai, S. H.: Effects of anesthetics and related drugs on the acetylcholine turnover rate in various structures of the rat brain. In Fink, B. Raymond (Ed.): Molecular Mechanisms of Anesthesia (Progress in Anesthesiology, Vol. 2). New York, Raven Press, 1980, pp. 189-198.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01506-06 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Narcotic analgesics and the regulation of catecholaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I:	L. Saiani	Visiting Fellow	SMRP	NIMH
Other:	K. Kumakura	Visiting Associate	SMRP	NIMH
	A. Guidotti	Chief	SMRP-NE	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Morphine increases striatal dopamine turnover rate. This increase is probably due to the stimulation of the opiate receptors located on DA nerve terminals of striatum. Morphine injected into substantia nigra(SN) failed to change striatal DA metabolism but blocked the activation of TH by haloperidol. This action of morphine was not reversed by bicuculline. Since opiate receptors in SN are located on neurons connecting SN with forebrain (possibly on substance P neurons), these results suggest that intranigral morphine prevents the haloperidol-induced activation of striatal TH by interfering with a non-GABAergic (perhaps substance P) projection to SN. The role of opiate receptors located on catecholaminergic cells was studied using primary cultures of adrenal chromaffin cells. These cells contain opiate receptors in measurable amounts. Stimulation of these receptors with agonists decreases the release of catecholamines elicited by nicotine. The results suggest that stimulation of opiate receptors modulates allosterically the function of nicotine and other receptors located on the chromaffin cell membranes.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study the action of narcotic analgesics administered acutely or chronically, on the function of various cells that release catecholamines. To establish whether narcotic analgesics interact with dopaminergic or catecholaminergic neurons at the pre- or postsynaptic receptor site, we studied changes in NE and DA turnover, in tyrosine hydroxylase activity (TH), of striatum, and in the release of catecholamines from primary cultures of cow adrenal chromaffin cells.

1. Experiments in striatum:

Analgesic doses of morphine and endorphins increased DA turnover and facilitated HVA accumulation in striatum. This effect was maintained after lesions of the cortico-striatal or striato-nigral pathway. However, after injection of morphine directly into SN (in doses of 1,2,5,10 and 20 μg of the SO_4 salt, dissolved in 0.5 μl saline) we found no change in striatal HVA content. These results suggest that unlike the opiate receptors in striatum, the opiate receptors in SN do not mediate the morphine-induced stimulation of striatal DA metabolism. Moreover, they suggest that opiate receptors are located on neurons other than dopaminergic and that these are phasic neurons. To gain further clarification of the process, we examined whether intranigral morphine could inhibit the activation of striatal TH induced by haloperidol. Perhaps, this activation is due to a phasic neuronal system stimulated by haloperidol. The action of haloperidol on striatal TH may be mediated via the transsynaptic regulation of DA neurons by nigral afferents, including the striato-nigral axons containing GABA or Substance P. We expected that if the nigral opiate receptors exerted an inhibitory influence on the DA neurons (either directly or indirectly), then, intranigral morphine would be able to antagonize the activation of striatal TH elicited by haloperidol.

Although intranigral morphine by itself did not change the affinity of striatal TH for $DMPH_4$, it completely prevented the increase in affinity of striatal TH for $DMPH_4$ in animals treated with haloperidol. This effect of intranigral morphine was reversed by systemic or intranigral injection of the opiate receptor antagonist, naltrexone, but was not reversed by intranigral injection of bicuculline methiodide, a GABA receptor antagonist. The specificity of the action of morphine was further supported by experiments which demonstrated the ability of muscimol to antagonize the haloperidol induced activation of striatal TH being reversed by intranigral bicuculline methiodide but not by naltrexone.

In order to determine whether this effect could be mediated by a direct action of morphine on DA cells in SN, we measured opiate receptor binding in SN after unilateral destruction of nigrostriatal DA neurons by 6-hydroxydopamine (6OHDA). Although 6OHDA treatment resulted in destroying more than 85% of the nigrostriatal DA neurons (based on TH measurements), no significant change was obtained in the binding of ^{3}H-enkephalinamide in crude P2 fractions of the lesioned SN. In contrast, there was a marked decrease (68%) in the binding of ^{3}H-enkephalinamide to SN P2 preparations from rats with surgical hemitransection of all the connections between striatum and SN. This lesion reduced both GABA and substance P content in SN by 60-85%.

The presence of opiate receptors on various neural elements of the nigral striatal loop supports the concept that stimulation of these receptors in selective portions of the loop will generate changes in DA metabolism, varying from excitation to inhibition. Striatal opiate receptors located on terminals of DA neurons are probably responsible for the increase of DA metabolites; however, the molecular mechanism for this increase is not clear. In SN, opiate receptors are not located on DA neurons and apparently are not involved in the control of basal DA function. However, when the DA system is activated by haloperidol, then the opiate receptors can reverse this action. Previous work in our lab has shown that substance P in SN may participate in the activation of TH by haloperidol. The experiments reported here suggest that Substance P neurons in SN may be regulated presynaptically by opiate receptors as they are in spinal cord. Thus, it appears that opiate peptides may have an additional neuromodulatory role in SN which could influence the activity of dopaminergic neural striatal projections. It remains to be established the nature of the SN axon terminals containing opiate receptors, the role of the opiates on the release of transmitters from these neurons, the type of opiate receptors, and the origin of these opiate peptides in SN.

2. Experiments in primary culture of adrenal chromaffin cells:

The primary culture of adrenal chromaffin cells from bovine adrenal medulla is an ideal model to study the molecular mechanisms by which the narcotic analgesics control the function of catecholamine containing cells. We have shown that chromaffin cells in culture contain high affinity, saturable stereospecific binding sites for opiate agonists and antagonists. The binding was characterized with ^{3}H-dihydromorphine, ^{3}H-D-Ala^2-leu-enkephalin, ^{3}H-ethylketazocine and ^{3}H-Sk&F 10047 to label the μ, δ, κ and σ receptors, respectively. ^{3}H-etorphine, which labels both μ and δ receptors, was also studied, as well as the two antagonists, ^{3}H-naloxone and ^{3}H-diprenorphine. The K_d was similar for all the compounds (approximately 2 nM).

A similar Kd was obtained when we studied the same ^{3}H-ligands in P2 membranes prepared from bovine frontal cortex. In brain, the Bmax for all the compounds ranged from 50 to 80 fmol/mg protein, whereas in adrenal medulla, the Bmax of the various compounds ranged from 20 to 200 fmol/mg protein. ^{3}H-etorphine, ^{3}H-naloxone, ^{3}H-diprenorphine and SK&F 10047 bind with a Bmax of 200, 150, 100, and 90 fmol/mg protein, respectively. ^{3}H-dihydromorphine, ^{3}H-D-Ala^2-leu-enkephalin and ^{3}H-ethylketazocine bind with a Bmax less than 20 fmol/mg protein.

In membranes of adrenal medulla, morphine, D-Ala^2-leu-enkephalin, beta-endorphin, naloxone, ethylketazocine and pentazocine displaced ^{3}H-etorphine (k_i = 100-400 nM), while diprenorphine, phenazocine and SK&F 10047 displaced ^{3}H-etorphine with a K_i of 4, 12 and 45 nM, respectively. The potency of morphine and D-Ala^2-leu-enkephalin for displacing ^{3}H-etorphine was greater in membranes prepared from adrenal medulla than in those prepared from frontal cortex.

Kinetic studies revealed that frontal cortex possesses two populations of receptors, while the adrenal medulla has only one labeled by ^{3}H-etorphine. ^{3}H-etorphine binding was not displaced by the nonopioid peptides, substance P, bradykinin, oxytocin, neurotensin, and cosyntropin; it was decreased by NaCl (100 mM) and GMP-P (NH) P (50 μM). These results suggested that etorphine receptors in bovine chromaffin cells are different from those in frontal cortex and that they are not of the μ, δ, or κ type. When these receptors are occupied by specific opiate agonists (etorphine, morphine, beta-endorphin, and FK 33824), the number of nicotinic receptors and the amount of catecholamines released by maximal doses of nicotine are reduced. The specificity of the action of opiate receptor agonists is supported by the ability of the opiate receptor antagonists to block their effects and by the inability of the opiate receptor agonists to reduce the Ca^{++}-independent release of catecholamines elicited by K$^+$ ions.

Conclusions

The opiate receptors are present in membranes of adrenal chromaffin cells. Activation of these receptors causes a non-competitive inhibition of the release of catecholamines elicited by the stimulation of nicotinic receptors. From our data, it can be inferred that when the met-enkephalin-like material in terminals of splanchnic nerve is released, it modulates the release of catecholamines induced by the stimulation of nicotinic receptors elicited by the concomitant neurally mediated release of acetylcholine. Since the Bmax for binding of the antagonist is large, chromaffin cells may contain two types of receptors, one specific for met-enkephalin and the other for beta-endorphin. Beta-endorphin released into the circulation from the intermediate or anterior lobe of the pituitary can influence the function of chromaffin cells.

The interrelations between the opiate agonists and the catecholamine secretion are relevant to the missions of the NIMH in many ways. Our present study may lead to a better understanding of the molecular synaptic mechanisms involved in the reciprocal control of catecholamines and enkephalin neuronal function, two functions involved in the etiology of affective disorders.

Publications

Guidotti, A., Moroni, F., Gale, K., and Kumakura, K.: Opiate receptor stimulation blocks the activation of striatal tyrosine hydroxylase induced by haloperidol. In Usdin, E. (Ed.): Catecholamine Basic and Clinical Frontiers, Pergamon Press, New York, pp 1035-1037, 1979

Kumakura, K., Guidotti, A., and Costa, E.: Primary cultures of adrenal chromaffin cells: modulation of nicotinic receptors by opiate receptor agonists. Nature 283: 489-492, 1980

Costa, E., Guidotti, A., Hanbauer, I., Saiani, L., Yang, H.-Y.T.: Regulation of cholinergic transmission in adrenal medulla. Proc. of International Symposium on Cholinergic Mechanisms, held in Florence Italy, 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01508-10 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Modulation by opiate and phencyclidine of GABAergic and cholinergic function in various brain areas.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Murray	PRAT Fellow	SMRP	NIMH
Other:	D.L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Subanesthetic doses of phencyclidine increase the turnover rate of acetylcholine in frontal and parietal cortex, but not in striatum or hippocampus when animals are sacrifieced 30 minutes after drug treatment. Phencyclidine-induced increase in cortical acetylcholine turnover rate was temporally correlated with the behavior effects of this compound. These results suggest that an activation of cortical cholinergic neurons may be related to these behavioral effects. In contrast to recent reports of specific ^{3}H-phenyclidine binding sites in rat brain, we demonstrated that this binding is actually an artifact of the rapid filtration assay. ^{3}H-phencyclidine binds to the glass fiber filters used in this assay in a manner resembling specific binding.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to evaluate the effects of opiates and phencyclidine on cholinergic and GABAergic neurons in various areas of the rat brain. These studies attempt to achieve a better understanding of the mode of action of phencyclidine and to provide information concerning the interactions among peptidergic neurons with opiate like activity, dopaminergic neurons and GABAergic neurons.

The turnover rate of acetylcholine and GABA were determined following infusion of phosphoryl-$^2H_9$-choline and ^{13}C-glucose, respectively. The percent of incorporation of the stable isotopic label into precursor (choline for acetylcholine and glutamate for GABA) and product was determined and the turnover rate was calculated by multiplying the fractional rate constant for product efflux by the steady state concentration of the product.

Following intraperitoneal administration of subanesthetic doses of phencyclidine, the peak behavioral effect occurs between 15 and 30 minutes after injection. The phencyclidine-induced behavioral syndrome exhibited during this time period is characterized by hypermotility, ataxia, and stereotypies. The concentrations of acetylcholine and choline were in steady state from 15 to 30 minutes after phencyclidine treatment in all brain areas studied. Phencyclidine increased the turnover rate of acetylcholine in the frontal and parietal cortex, but not in the striatum or hippocampus when the animals were sacrificed 30 minutes after drug treatment. Thus, phencyclidine-induced increase in cortical acetylcholine turnover rate is temporally correlated with the behavioral effects of this compound. These results suggest that an activation of cortical cholinergic neurons may be related to the behavioral effects of phencyclidine.

The interaction of ^3H-phencyclidine with high affinity binding sites on rat brain membranes was examined in an attempt to determine the role of these receptors in the above mentioned effects on acetylcholine utilization. However, in contrast to recent reports of a specific ^3H-phencyclidine binding site, we demonstrated that this binding was actually an artifact of the rapid filtration assay. We found that ^3H-phencyclidine binds to the glass fiber filters used in this assay in a manner resembling specific binding. Thus, the spurious specific binding of this ligand to the filters renders the rapid filtration assay unsuitable for ^3H-phencyclidine binding studies. Current experiments are attempting to demonstrate specific ^3H-phencyclidine recognition sites using other methods of separation of bound and free ligand.

In rats, morphine (s.c.) and beta-endorphin (i.v.t.) administration decreases the turnover rate of gamma-aminobutyric acid (GABA) in the N. caudatus, but increases the turnover rate of GABA in globus pallidus and substantia nigra. The effects of morphine on the turnover rate of GABA are reversed by naltrexone. There is no dopaminergic involvement in the turnover rate of GABA. The increase of GABA turnover in globus pallidus seems to play an important role in the morphine-induced catalepsy. In fact, muscimol, a GABA receptor stimulating agent, when injected bilaterally into the globus pallidus mimics morphine-induced catalepsy. Thus, the action of morphine and beta-endorphin on the turnover rate of GABA in the caudate and globus pallidus may be due to an effect on opiate receptors located in the head of the caudate nucleus.

The recent documentation of a phencyclidine-induced psychosis associated with the illicit use of this compound is of particular interest due to its resemblance to acute schizophrenia. Thus, an understanding of phencyclidine's mode of action might provide insight into the mechanisms underlying psychotic states. To this end, we plan to investigate the locus and mechanism(s) in the brain which mediate the phencyclidine-induced increase in cortical acetylcholine metabolism. In addition, the role of cholinergic and GABAergic function in the development of tolerance to phencyclidine will be evaluated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01509-10 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Pharmacological studies of acetylcholine turnover: effects of diet and stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	N. Brunello	Visiting Associate	SMRP
Other:	D.L. Cheney	Chief	SMRP-MP

COOPERATING UNITS (if any)

LAB/BRANCH
Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The turnover rate of acetylcholine was studied in animals exposed to different environmental conditions for different periods of time. Exposure to cold for 1 hour selectively decreased the turnover rate of acetylcholine in the hypothalamus and frontal cortex. A long period of immobilization failed to change the indices of cholinergic activity. Rats were given diets with different choline contents, the turnover rates of acetylcholine in brain areas of rats fed with a choline free diet were unaffected whereas the levels of choline were slightly decreased. In rats maintained on a choline rich diet we found a 40% decrease in the turnover rate of hippocampal acetylcholine.

PHS-6040
(Rev. 10-76)

Project Description:

The present studies were concerned with the role of stress and the role of different dietary conditions in the regulation of cholinergic neurons of CNS. Experimental details have been described in Project No. Z01-MH-01509-07 SMRP. To inject the deuterated phosphorylcholine, the labeled precursor of choline, in the stress experiments, a chronic cannula was inserted in the jugular vein 3 days before the experiment. The cannula emerged from the skin behind the head and was long enough to allow the rat to move freely inside its cage. To study the effects of diets on the indices of cholinergic activity, diets with different contents of choline were given for at least one month.

Stress: Catecholamines have been singled out as the main mediators of stress; since in brain various neuronal pathways interact transsynaptically, it is conceivable that catecholamines mediate stress by modifying other neurotransmitter function. The effect of cold exposure on the turnover rate of acetylcholine was studied by exposing the rats one per cage with wet fur to 4° for various time periods. Simultaneously with a decrease in body temperature, at early times (20-60 minutes) there was a significant decrease in acetylcholine turnover rate in hippocampus and hypothalamus, the effect being restricted to these areas without any change in the steady state levels of ACh in neither of the brain areas tested (frontal and parietal cortex, striatum, hypothalamus and hippocampus). Another stressful condition, such as longer periods of immobilization failed to change these indices of cholinergic activity.

Diet: Several studies have indicated that the availability of the precursor of acetylcholine is an important factor regulating the dynamics of acetylcholine metabolism. In order to evaluate whether a greater availability of choline could influence the turnover rate of acetylcholine in rat brain, animals were kept on diets at different contents of choline. Some were given a control diet (0.2% Ch), others a choline rich diet (2.02% Ch),and still other a choline free diet (0.01% Ch) for at least one month. Acetylcholine content was unchanged in all brain areas tested and the levels of choline were slightly decreased in the animals maintained on a choline free diet. In both frontal and parietal cortex and in the caudate there was no change in the turnover rate of acetylcholine in the three groups of animals, whereas there was a 40% decrease in the turnover rate of hippocampal acetylcholine in rats maintained on a choline rich diet. Our results suggest that the steady-state concentration of brain acetylcholine is maintained constant within narrow physiological limits and that further availability of exogenous choline may not be crucial in the regulation of acetylcholine synthesis. In some extreme cases, the turnover of acetylcholine may be decreased when the choline stimulates the cholinergic receptors of a system with feedback inhibition regulation.

Publications:

Wood, P.L., Cheney, D.L., and Costa, E.: An investigation of whether septal gamma-aminobutyrate-containing interneurons are involved in the reduction in the turnover rate of acetylcholine elicited by substance P and beta-endorphin

in the hippocampus. Neuroscience 4: 1479-1489, 1979

Wood, P.L., Cheney,D. L., and Costa, E.: A prolactin action on acetylcholine metabolism in striatum, hippocampus and thalamus. J. Neurochemistry 34: 1053-1057, 1980

Costa, E., Tagliamonte, A., Brunello, N., and Cheney, D.L.: Effects of stress on the metabolism of acetylcholine in the cholinergic pathways on extrapyramidal and limbic systems. In: Kvetnansky, R. (ed.): Catecholamines and Stress: Recent Advances, Elsevier/North Holland Publ., in press

Wood, P. L., Cheney, D. L., and Costa, E.: Interactions of neuropeptides with cholinergic septal hippocampal pathway: induction for a possible transsynaptic regulation. In Pepeu, G. (ed.): Cholinergic Mechanisms, Oxford, Pergamon Press, in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01510-05 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of cannabinoids on cholinergic and GABAergic dynamics in rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Murray	PRAT Fellow	SMRP	NIMH
Other:	A. V. Revuelta	Visiting Associate	SMRP	NIMH
	D. L. Cheney	Chief	SMRP-M	NIMH

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LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The tetrahydrocannabinoids, (-)-delta-8-THC-DMH, (-)-delta-9-THC, and (-)-delta-8-THC, specifically reduce the turnover rate of acetylcholine in the hippocampus and the turnover rate of GABA in septum in a dose-dependent manner. The (+) isomer of delta-8-THC fails to alter these parameters. These results suggest that the tetrahydrocannabinols are activating specific receptor binding sites probably in the septum. However, this high degree of nonspecific binding of ^{3}H-delta-8-THC due to its high lipid solubility precludes the use of a rapid filtration method with glass fiber filters to document the presence of specific recognition sites for these compounds.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of this research project was to determine the effects of delta-9-THC and related compounds on the dynamics of the cholinergic and gamma-aminobutyric acid (GABA)ergic neurons in rat brain.

The turnover rate of acetylcholine and GABA were determined following infusion of phosphoryl($^2H_9$)choline and ^{13}C glucose, respectively. The percent of incorporation of the stable isotopic label into precursor (choline for acetylcholine and glutamate for GABA) and product was calculated. From the interrelationship between the precursor and product, the fractional rate constant for product efflux can be estimated. The turnover rate can be obtained by multiplying the fractional rate constant for product efflux by the steady state of the product.

We have compared the effects of (-)-delta-8-tetrahydrocannabinol (THC), (+)-delta-8-THC, and the dimethylheptyl (DMH) homolog of (-)-delta-8-THC with the action of (-)-delta-9-THC on the turnover rate of acetylcholine and GABA in various brain areas. We found that (-)-delta-8-THC-DMH, (-)-delta-9-THC and (-)-delta-8-THC all specifically reduce the turnover rate of acetylcholine in the hippocampus and the turnover rate of GABA in septum in a dose-dependent manner. The (+)-isomer of delta-8-THC fails to change either the turnover rate of acetylcholine or GABA. Perhaps, the tetrahydrocannabinoids are activating specific receptors (binding sites) which indirectly modulate the activity of the cholinergic neurons in the septal hippocampal pathway. The notion implies that the binding of cannabinoids to these sites would mediate the characteristic effects of these compounds.

We attempted to test this hypothesis by direct labeling of the sites with ^3H-(-)-trans-delta-8-tetrahydrocannabinol using a rapid filtration method of separating bound from free ligand. The binding of ^3H-delta-8-THC to membrane preparations of rat brain did not appear to be saturable over a wide concentration range of ligand and tissue. The observed binding of ^3H-delta-8-THC was characteristic of a non-specific partitioning of the ligand into the membrane phase. The nature of this binding was not altered by employing a variety of buffer systems or vehicles for the cannabinoids. Furthermore, the binding of ^3H-delta-8-THC was not displaceable by unlabeled delta-9-THC or cannabidiol. A troublesome artifact of increased ^3H-delta-8-THC binding to glass fiber filters in the presence of unlabeled delta-9-THC or cannabidiol was revealed when non-tissue controls were performed in the filtration assay. Thus, the high degree of nonspecific binding of ^3H-delta-8-THC due to its high lipid solubility precludes the use of a rapid filtration method with glass fiber filters to determine a specific binding component of this ligand. Cannabinoids of a higher specific activity than employed here and different methods for the separation of bound from free ligand will be required to document the existence of specific recognition sites for these compounds.

From the biomedical point of view this investigation can help us to better understand the mechanism whereby delta-9-THC and other related analogues of the widely used cannabis sativa act to modify mood and perception.

Publications:

Revuelta, A. V., Cheney, D. L., Costa, E., Lander, N., and Mechoulam, R.: Reduction of hippocampal acetylcholine turnover in rats treated with (-)-delta-8-tetrahydrocannabinol and its 1',2'-dimethyl-heptyl homolog. Brain Res., in press.

Cheney, D. L., Revuelta, A. V., and Costa, E.: Marijuana and cholinergic dynamics. In Pepeu, G. (Ed.): Cholinergic Mechanisms. Oxford, Pergamon Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01511-07 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Neuronal monoamines: a study of their integration and regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief	SMRP-N	NIMH
Other:	F. Vargas	Visiting Fellow	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Lab. Preclinical Pharmacology

SECTION
Section on Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The integrated actions of dopaminergic neurons were investigated in striatum and N. accumbens after short and long term treatment with antischizophrenic drugs. Long term treatment with classical antischizophrenics produce tolerance to the activation of the DA striatal system. Tolerance is not observed after long term treatment with atypical antischizophenics. In striatum, the supersensitivity of DA receptors produced by these drugs is associated with an increase in calcium binding protein. Th calmodulin content was determined in striatum and frontal cortex of control subjects and in eleven schizophrenic patients. No differences were observed in the calmodulin content between these two groups.

instance, in N. accumbens, but not in striatum, the GABAergic neurons are innervated by dopaminergic neurons. We plan to determine the precise role of the striato-nigral pathway and striatal and accumbens interneuronal connections in the regulation of DA neuronal function. In particular, we are eager to study the respective influence of nigra and striatal substance P, GABA, and opiate peptides or opiate agonists and antagonists in the regulation of DA activity. In addition, we plan to study the role of cortico-striatal glutamatergic projections. According to general clinical experience the full therapeutic response to a neuroleptic agent may require several weeks and may last for a considerable time after cessation of therapy. There are reasons to believe that the primary actions of neuroleptics, as observed in short term experiments, is only the first response to induce a series of secondary effects, the nature of which has not been fully clarified. We propose to extend our study to the different neuronal systems associated with the DA system and to long term treatment with classical and atypical antipsychotics.

B. Calmodulin content in corpus striatum and frontal cortex of schizophrenics

Long term treatment with classical neuroleptics in addition to the tolerance to the increase of striatal TH elicited by these drugs, generates supersensitivity to the behavioral effects of apomorphine. The supersensitive response of DA receptors is associated with an increase in the striatal content of Ca^{++} binding protein (calmodulin). Based on this knowledge, we reasoned that if calmodulin was fundamental to the processes involved in dopaminergic neurotransmission, then alteration of calmodulin system could be expected in those abnormalities of dopaminergic neurotransmission associated with the behavior disorders of schizophrenia. Therefore, we undertook the study of the calmodulin content in caudate and cerebral cortex from autopsied brain of normal and schizophrenic individuals. We were unable to find significant differences between calmodulin content measured in membranes obtained from striatum or cortex of nine control subjects without a psychiatric history and from seven schizophrenic individuals.

Since the impairment of DA transmission and the increase of striatal ^{3}H-neuroleptic binding has been reported in schizophrenia, a change in calmodulin content might have been expected. However, it is interesting to note that just as there is no increase of calmodulin, there is also no increase of DA sensitive adenylate cyclase in schizophrenic brain. We should conclude that the calmodulin in striatum of schizophrenic patients does not play a role in the control of DA responses. Alternatively, we have to bear in mind that the schizophrenic patients' brain tissue that we analyzed was subjected to chronic neuroleptic treatment at some time before the patient died. Recently, it was reported that chronic neuroleptic drugs may induce an increase in membrane bound calmodulin concentrations; an effect that persists for a long time after cessation of drug treatment. Thus, it is not inconceivable to suppose that calmodulin content in brain of schizophrenic patients was low but it returned to a normal level as a consequence of neuroleptic treatment.

Project Description:

Although the catecholamine hypothesis of affective disorders has received support during the past decade, today it is evident that catecholamines are not the only transmitter system involved in the pathogenesis of affective disorders. In fact, all aspects of the pathophysiology of various forms of affective disorders cannot be explained by simple alterations of catecholamine metabolism; it is clear, however, that many other concomitant biochemical, physiological, and psychological factors must be involved.

During the past years, many studies have demonstrated the complex anatomical, physiological and biochemical interactions among monoaminergic and other neuronal systems. In this project, we studied the consequences of short- and long term treatment with neuroleptics endowed with antischizophrenic activity on the dopaminergic neurons and on the psotsynaptic striatal events that follow the stimulation of DA receptors.

A. Consequences of short and long term treatment with neuroleptics on DA neurons

1. Antischizophrenic drugs that elicit a high incidence of extrapyramidal side effects, termed "classical," preferentially increase dopamine turnover in striatum. The antischizophrenics that cause a low incidence of extrapyramidal side effects, termed "atypical," preferentially increase the turnover of DA in N. accumbens.

2. Long-term treatment with classical (cataleptogenic) and atypical (noncataleptogenic) neuroleptics produces differential effects on TH activity of locus coeruleus, C. striatum and N. accumbens. The activity of TH (Vmax) in locus coeruleus but not in striatum or nucleus accumbens is increased in rat treated for a long period (10 to 90 days) with 0.5 mg/kg/day of haloperidol.

3. After chronic haloperidol treatment, the drug is withdrawn for 3,15, or 30 days, challenging doses of haloperidol or other classical neuroleptics (pimozide, chlorpromazine, methiotepine) fail to increase the affinity of striatal TH for $DMPH_4$. Such tolerance to haloperidol was also present in N. accumbens; however, in this tissue the tolerance disappears seven days after haloperidol withdrawal. After chronic haloperidol treatment there is no cross tolerance with clozapine or sulpiride-induced increase of striatal TH affinity for $DMPH_4$.

4. Tolerance to long-term treatment with classical neuroleptics was not prevented by long term treatment with Li++. The rat treated chronically with clozapine and sulpiride showed no tolerance to the effects on TH when given challenging doses of clozapine or haloperidol.

Several reasons account for the differential development of tolerance and supersensitivity to the stimualtion of TH in striatum and N. accumbens: among others, a different synaptic wiring in the two nuclei could play a role. For

Obviously, it would be desirable to measure calmodulin content in the brain of schizophrenic patients not subjected to drug treatment. To collect striata of schizophrenic patients not subjected to neuroleptic treatment and to measure calmodulin content in this tissue is the goal of our future research.

Publications:

Guidotti, A.: Regulation of tyrosine hydroxylase after chronic treatment with classical and atypical antischizophrenic drugs. In: F. Cattabeni, R. Racagni, and P.F. Spano (eds): Advances in Biochemical Psychopharmacology, New York, Raven Press, in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01512-07 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Transmitter interactions in the regulation of pituitary function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief	SMRP-N	NIMH

COOPERATING UNITS (if any)

L. Grandison, Dept. Physiology and Biophysics, College of Medicine and Dentistry, Rutgers Medical School, Piscataway, N.J.

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of GABA, catecholamine and endorphins in the response of hypothalamus to different psychoactive drugs was studied by monitoring pituitary hormone release. Endogenous opiates stimulate PRL and block FSH release by activation of hypothalamic opiate receptors. These opiate receptors may be located on cells containing prolactin releasing factor since median eminence DA neurons or serotoninergic neurons apparently do not mediate the effects of morphine. Stimulation of hypothalamic or brain GABA receptors with muscimol fails to block morphine or haloperidol-induced PRL release. In contrast stimulation of GABA receptors in anterior pituitary prevents the morphine or haloperidol-induced PRL release.

PHS-6040
(Rev. 10-76)

Project Description:

Hypothalamic neuronal mechanisms transsynaptically regulate behavior and secretion of polypeptide releasing factors (RF's). The RF's, in turn, regulate pituitary function by stimulating adenylate cyclase in the anterior pituitary. In order to define the type of neurotransmitters involved in the release of pituitary hormones, the effects of neuroactive drugs were monitored by the measurement of pituitary hormone release.

1) Effect of opiate receptor agonists and antagonists

Injection of β-endorphin into the basomedial hypothalamus induced an increase of PRL release. Naltrexone was able to block this response. Specificity was further demonstrated since a 10-fold higher dose of α-endorphin (inactive peptide) was ineffective. When injected into the hypothalamus naltrexone itself produced a suppression of PRL release. After deafferentation morphine still stimulated and naltrexone still inhibited PRL release. In contrast to its activity in vivo, β-endorphin ($2x10^{-8}M$; $2x10^{-7}M$) or naltrexone ($10^{-4}M$) had no effect on PRL release from the pituitary in vitro. In addition, β-endorphin was unable to alter the inhibitory action of $5x10^{-7}M$ apomorphine on *in vitro* pituitary halves.

Next, we attempted to determine the events after stimulation of hypothalamic opiate receptors that induce PRL release. When the DA tonus was potentiated by giving pargyline (a monoaminoxidase inhibitor), PRL release following submaximal doses of morphine or β-endorphin was not altered. In contrast, when the DA activity was reduced by administration of reserpine or haloperidol, pargyline significantly reduced the increase release of PRL. In addition, the action of morphine or β-endorphin but not that of reserpine or haloperidol was blocked by naltrexone. Pretreatment of the animals with PCPA in doses that significantly inhibit tryptophan hydroxylase activity or intrahypothalamic injection of muscimol (a GABA mimetic) did not alter morphine induced PRL release.

2) Effect of GABA receptor agonists

The rise in serum prolactin (PRL) concentration after haloperidol (0.05 to 0.5 mg/kg i.v.) or morphine (2 to 16 mg/kg s.c.) in male rats and during estrogen treatment (1 mg/day/15 days) in ovariectomized rats was reduced by systemic injection of the GABA receptor agonist muscimol (1 mg/kg i.v.). However, intraventricular or intrahypothalamic injection of muscimol (100-400 ng) was ineffective in blocking PRL release induced by these compounds.

Since the distribution and concentration of muscimol in brain after central administration was similar to that following systemic injection, these observations demonstrate that stimulation of central GABA receptors was not involved in the regulation of PRL release during these processes. Rather the high levels of muscimol in the anterior pituitary after systemic injection and its absence after central administration suggested that blockade of PRL release by muscimol result from a stimulation of GABA receptors located in the pituitary. Indeed, ^{3}H-muscimol and ^{3}H-GABA receptor recognition sites with characteristics similar to that of brain were present in rat anterior pituitary. In addition, muscimol (10^{-8} to 10^{-6}M) reduced the spontaneous release of PRL from incubated anterior pituitary halves in vitro. This effect was reversed by the GABA receptor antagonist, bicuculline, but not by the dopamine antagonist, sulpiride. These results provide evidence that GABA receptor stimulation in the anterior pituitary can act to regulate the release of PRL. These data suggest that GABA mimetic drugs which fail to enter the brain may represent a relevant approach to control the PRL-releasing action of neuroleptics without altering their central effects.

These data suggest that catecholamines, GABA and endorphins are all involved in regulation of anterior pituitary functions. From these studies it appears that different neurotransmitter systems are involved in the release of specific anterior pituitary hormones. For example, PRL release is under hypothalamic DA and endorphin control but not under a direct hypothalamic GABA control. GABA might control PRL release acting directly at the pituitary level independently from catecholamines. Very recently, we observed that GABA receptors are present in human anterior pituitary. Further efforts are now required to extend these studies to the numerous hypothalamic peptide neurotransmitter substances (neurotensin, somatostatin, substance P, bombesin) which have been shown to influence hormone release. These observations and the demonstration of large concentrations of GABA in human anterior pituitary suggest a possible physiological role of pituitary GABA receptors in the control of PRL release. In addition, if PRL release can be controlled by stimulation of GABA receptors located on the anterior pituitary, peripheral GABA-mimetic drugs that do not enter the brain may represent a relevant approach to control of the PRL-releasing action of the neuroleptics without altering their control effects.

These studies are relevant to understanding total brain function because the hypothalamic neuronal peptides investigated here are now known to be widely distributed throughout the brain. The hormone release neurotransmitter function relationship in the hypothalamus-pituitary axis may be used as a tool to learn the action of different psychoactive drugs on neuropeptidergic transmission.

Publications:

Grandison, L., and Guidotti, A.: Gamma-aminobutyric acid function in rat anterior pituitary: Evidence for control of prolactin release. Endocrinology 105: 754-759, 1979.

Grandison, L., Fratta, W., and Guidotti, A.: Location and characterization of opiate receptors regulating pituitary secretion. Life Sci. 26: 1633-1642, 1980.

Grandison, L., and Guidotti, A.: Endorphin stimulation of prolactin release: Its relationship to median eminence DA neurons. In Usdin, E., Kopin, I. J. and Barchas, J. D. (Eds.): Catecholamines: Basic and Clinical Frontiers. Oxford, England, Pergamon Press, 1980, pp. 1242-1344.

Grandison, L., and Guidotti, A.: Role of anterior pituitary GABA receptor in the control of prolactin release. In Muller, E. (Ed.): Neuroactive Drugs in Endocrinology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01514-08 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Trans-synaptic control of protein synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	L. Saiani	Visiting Fellow	SMRP
Other	A. Guidotti	Chief	SMRP-N
	E. Costa	Chief	SMRP

COOPERATING UNITS (if any)

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In primary culture of chromaffin cells from cow adrenal medulla TH induction is preceded by an activation of cytosol cAPK and by an increase in nuclear protein phosphorylation. Both the induction of TH and the increase of nuclear phosphorylation require that the assembly of microtubular proteins be functional. Antimicrotubular drugs such as colchicine and vinblastine (10^{-9}M) can block the TH induction elicited by 8-Br-cAMP, when the drugs are added less than 15 hours after 8-Br-cAMP. Since colchicine, added within cAMP also prevents the increase in nuclear phosphorylation, it is possible that the assembly of microtubular proteins might be operative in the intracellular translocation and nuclear uptake of catalytic subunits of cAPK activated by the addition of 8-Br-cAMP. In addition, these data support the view that an increase in nuclear protein phosphorylation is an essential step in the mediation of the acceleration of mRNA synthesis and the subsequent increase in TH synthesis elicited by 8-Br-cAMP.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study the molecular mechanisms whereby transsynaptic stimuli induce new synthesis of specific proteins in chromaffincells of adrenal medulla. In previous studies we reported that in chromaffin cells of rat adrenal medulla, the sequence of molecular events whereby transsynaptic mechanisms regulate the genetic code expression includes an increase in the cAMP/cGMP concentration ratio, an activation of cAMP-dependent protein kinase (PK) in cytosol and the translocation of the low molecular weight catalytic subunit of this protein kinase from the cytosol to the subcellular particles. The PK of nuclei is not regulated by cAMP but it increases during the transsynaptic induction of tyrosine-3-monooxygenase (TH) because the cAPK catalytic subunits translocate from cytosol to the nucleus. Thus, the activation and translocation of PK, triggered by the initial increase of cAMP due to the activation of cAPK acts as a long range messenger for the transsynaptic expression of the genetic code.

An injection of nicotinic receptor antagonist or the denervation of adrenal medulla reduces and shortens the cAMP elevation caused by cold exposure and prevents the cAPK activation; it inhibits the nuclear translocation of cAPK catalytic subunits and also blocks the induction of TH. Though these experiments demonstrate a temporal succession between the translocation of the catalytic subunits of cAPK to the nucleus, the increase in nuclear protein phosphorylation and the stimulation of gene activity leading to an increase in TH synthesis, they fail to elucidate: 1) the specific regulatory mechanism involved in the nuclear uptake of catalytic subunits of cAPK; 2) the precise role in nuclear function of the subunits of cAPK that translocate to the nucleus; 3) the mechanisms underlying gene transcription specificity; and 4) the necessity of nuclear translocation of cAPK catalytic subunits in eliciting the transsynaptic induction of TH.

In vivo experiments with rat adrenal medulla are not the most suitable means to study transsynaptic regulation of gene expression at the molecular level because rat adrenal medullae provide only a limited amount of purified nuclear material which does not allow a rigorous monitoring of the fine biochemical events occurring in the nuclear chromatin.

We have then decided to use as an in vitro system primary cultures of cow adrenal medulla cells. These cells were used as a model to evaluate the ability of 8-Br-cyclic AMP (8-Br-cAMP) to induce TH and to study the role of cAPK in this induction. This cell culture maintains a constant level of cyclic nucleotides, catecholamines and related enzyme activities for about four weeks.

Exposure of the cells for 3 hrs to 8-Br-cAMP produces 48 hrs later, a dose related longlasting increase in TH activity; 8-Br-cGMP fails to modify TH. The increase in TH activity caused by 8-Br-cAMP is due to an increase of the Vmax and is preceded by an activation of cytosol cAPK associated with a decrease of the total cytosol cAPK. A sustained increase in nuclear phosphorylation begins 8 to 12 hrs after 8-Br-cAMP application. The delayed increase in TH activity induced by 8-Br-cAMP is blocked by actinomycin D, cycloheximide, colchicine and vinblastine. This reduction of TH induction elicited by colchicine and vinblastine (10^{-9}M) is observed only when these inhibitors of the microtubular protein polymerization were added 4 to 12 hrs after the addition of 8-Br cAMP which is the inducing stimulus. The addition of colchicine 15 hrs after 8-Br cAMP fails to inhibit TH induction. This blockade of TH induction is associated with an inhibition of the increase in nuclear phosphorylation, but is not associated with an inhibition of protein synthesis. The increase of endogenous cAMP and the induction of TH were also produced by cholera toxin. These results suggest that the increase of TH elicited by 8-Br-cAMP is mediated by the translocation of cAPK subunits from cytosol to the nuclei and that this translocation requires the function of the microtubular network. In future studies, we plan to study how the increase in nuclear phosphorylation regulates the expression of the genetic code during the TH induction.

Since in adrenal medulla, the availability of catecholamines and the adrenergic function are regulated by TH and since adrenergic mechanisms have been implicated in the etiology of affective disorders, an understanding of the molecular nature of the regulation of the biosynthesis of catecholamines may contribute to a better understanding of the synaptic defects that may be operative in the etiology of mental diseases. In addition, the translocation of cAPK subunits may operate as a basic mechanism in memory and/or learning.

Publications:

Kumakura, K., Guidotti, A. and Costa, E.: Role of protein kinase translocation in the induction of tyrosine-3-monooxygenase (TH) in adrenal chromaffin cell cultures. In: Usdin, E. (ed.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, pp 61-63, 1979

Kumakura, K., guidotti, A. and Costa, E.: Primary cultures of chromaffin cells: molecular mechanisms for the induction of tyrosine hydroxylase mediated by 8-Br-cyclic AMP. Molecular Pharmacology 16: 865-876, 1979

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01515-07 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Short and long term biochemical changes in cerebellum after treatment with psychoactive drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Mazzari	Guest Worker	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biochemically, the cerebellum is characterized by a large concentration of GABA, cAMP, cGMP-dependent protein kinase and a heat-stable protein which inhibits the cGMP-dependent protein kinase. Drug-induced changes in GABA receptor function can be easily monitored by measuring changes in these biochemical parameters. It is suggested that diazepam and muscimol (an indirect and a direct GABA receptor agonist), modulate cerebellar phosphorylation by altering the relationship between cGMP-dependent protein kinase and the content of the cerebellar inhibitor of protein kinase. They could act on the content of the protein kinase inhibitor by decreasing the cerebellar cGMP content without modifying that of cAMP. Since very recently we have established that the endogenous inhibitor of protein kinase is identical to the inhibitor of GABA receptor binding (GABA-modulin), the cerebellum became the organ of choice for studying the relationship between GABAergic function, phosphorylation of selective membrane proteins and GABA-modulin content.

PHS-6040
(Rev. 10-76)

Project Description:

The cerebellum is the brain region where the synaptic interactions among various neurons has been determined with greater detail and precision. The project is designed to relate changes of cAMP, cGMP content and protein kinase activities with the release of transmitters in the cerebellar cortex and deep cerebellar nuclei. Such studies are possible in cerebellum because:

1. The climbing fibers and the mossy fibers are the two major excitatory inputs to the Purkinje cells.

2. The function of Purkinje cells is regulated by inhibitory (GABAergic) interneurons.

3. The Purkinje cells are the sole input from the cerebellum and release GABA on the deep cerebellar neurons.

In previous reports we have shown that pharmacological manipulation of climbing or mossy fibers not only change the firing pattern of Purkinje cells but also increase the cGMP content of the cerebellar cortex. Similarly, manipulation of the cerebellar GABAergic function changes the firing rate of the Purkinje cells and the cerebellar cGMP content. When the concentration of cerebellar GABA is increased or GABA receptors are stimulated, the firing rate of Purkinje cells and the cGMP content of cerebellar cortex are reduced. Conversely, when GABA receptors are blocked, the firing rate of Purkinje cells and the cerebellar cGMP content are increased.

These results suggest that two excitatory transmitters released by the parallel or climbing fibers and GABA released by cerebellar interneurons are independently involved in changing cGMP content on the molecular layer of cerebellum.

As we have previously reported (Szmigielski, A., Guidotti, A., Costa, E. J. Biol. Chem. 252: 3848-3853, 1977) the rat cerebellum contains a significant amount of cGMP-dependent protein kinase and a large concentration of protein kinase inhibitors. These inhibitors are thermostable proteins which can be separated by gel chromatography into 2 molecular forms: the Type I and Type II inhibitor of protein kinase. The Type I inhibitor blocks the rat cerebellar cGMP-dependent protein kinase activity while the Type II inhibitor blocks the rat cerebellar cGMP-dependent protein kinase and the cyclic nucleotide independent protein kinase. The activity of the Type II inhibitor varied inversely with changes of cerebellar cGMP content after injection of harmaline or diazepam. No changes of Type I inhibition were observed under these conditions.

The drug-induced shift of Type II inhibitor of protein kinase was not mediated by changes in protein synthesis because it persisted after pretreatment with cycloheximide. These results are compatible with the hypothesis that cGMP modulates phosphorylation in cerebellum by changing the relationship between cGMP-dependent protein kinase and Type II inhibitor content. Experiments were then directed to elucidate the possible message mediated by the increase of cGMP in the cerebellum. To achieve this goal our attention has been focused on cGMP dependent protein kinase and on the thermostable protein inhibitors of protein kinase-dependent phosphorylation. We have developed a simple method to assay cGMP-dependent protein kinase which allows the simultaneous processing of a large number of samples. Using this method we have established that cerebellar cGMP-dependent protein kinase is very low at birth and increases with age in coincidence with several ontogenetic events in cerebellum. One of the events that more closely relates to the increase of cGMP-dependent protein kinase, is the formation of a permanent synaptic contact between climbing fibers and Purkinje cells. In addition using mutant mice (nervous, weaver, staggerer) and rats treated with 3-acetylpyridine we further established a fundamental role of intact climbing fiber-dendritic synaptic contacts for the maintanence of normal cGMP-dependent protein kinase activity in cerebellum.

Considering the important role of the cerebellum in the regulation of the sensory motor cortex, function, and the large abundance of inhibitory (GABA, taurine) or excitatory transmitters (aspartic and glutamic acid) it is conceivable that studies on the modification of the cerebellar cGMP system may become an important tool for the study of the action of neuroleptic and antidepressant drugs.

Our proposed course is to define the molecular nature of the messages mediated by the increase of cGMP. We have now established that rat cerebellum contains (1) a large amount of cGMP-dependent protein kinase and (2) high concentrations of a thermostable small molecular protein which inhibits cAMP-dependent and cGMP-dependent protein kinase. In addition, we have established that the protein inhibitor of protein kinase is identical to the modulator of GABA receptor binding.

We intend to study whether the transmitter-mediated changes of cerebellar cGMP-dependent protein kinase are paralleled by changes of GABA receptor binding and by changes in the content of the endogenous protein inhibitor of the protein kinase and of GABA binding.

Publication:

Bandle, E., and Guidotti, A.: A simple and rapid method for the assay of cyclic AMP-dependent protein kinase. J. Neurosci. Methods, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01516-07 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical pharmacology of minor tranquilizers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Baraldi	Visiting Associate	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH
	D. Konkel	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The molecular mechanism whereby GABAergic transmission is modified by benzodiazepines (BDZs) was studied in vivo and in vitro. In vivo BDZs like the GABA mimetic, muscimol antagonize the isoniazid-induced convulsions, and the isoniazid-induced increase of cerebellar cGMP and pituitary cAMP content. In vitro BDZs facilitate binding of GABA to its receptor sites by competing with the endogenous membrane protein (GABA-modulin) which acts as an inhibitor of high affinity Na^+-independent GABA binding to synaptic membrane preparations. GABA-modulin competes with BDZs for their specific receptor sites. GABA, benzodiazepine receptors and GABA-modulin are present in the membranes of a neuroblastoma cell line. In these cells stimulation of GABA receptors increases Cl^- influx and BDZs facilitate the action of GABA. This increase in the binding affinity of GABA for its postsynaptic site may represent the molecular mechanism by which BDZs facilitate GABAergic transmission in vivo. These observations may help in elucidating details of the supramolecular nature of postsynaptic GABA and BDZ receptors and also may offer a way to study the biochemical defects in anxiety states.

PHS-6040
(Rev. 10-76)

Project Description:

Recent evidence from this laboratory has suggested that benzodiazepines, which are the most widely prescribed drug, may exert a beneficial effect in psychiatric disturbance by a primary action on GABA mechanisms. The present project intends to present new behavioral evidence which favor a mediation of GABA in the anxiolytic action of benzodiazepine and intends to elucidate the molecular mechanisms of action of benzodiazepines.

BDZs like muscimol (a direct GABA receptor agonist) and intraventricular nipecotic acid (a blocker of GABA uptake) delayed the onset of convulsions induced by isoniazid in doses that did not protect the animals from strychnine convulsions. Moreover, diazepam and muscimol had a similar potency in preventing the increase of cerebellar cGMP or of pituitary cAMP induced by isoniazid, picrotoxin, harmaline and reserpine. In the nigrostriatal system BDZs in a manner similar to GABA receptor agonists, block the activation of DA neurons induced by antipsychotics.

The increase in punished behavior elicited by benzodiazepines in rats operating in a conflict test situation is potentiated by intraventricular injections of muscimol. Muscimol, injected intraventricularly in doses of 50 to 200 ng also produces a benzodiazepine-like action by increasing the output of punished operating behavior. These data concur with the hypothesis that the pharmacological action of BDZs may be mediated by an effect on GABA neurons.

To examine at the molecular level whether BDZs changed the binding of ^{3}H-GABA to postsynaptic receptors, we measured whether BDZs changed the binding of GABA to freshly prepared synaptic plasma membranes or to membranes treated with Triton X-100 frozen and thawed repeatedly. BDZs appear to unravel a high affinity component for the binding of GABA to fresh membranes but fail to change the GABA binding to frozen and thawed synaptic plasma membranes. Similar results were obtained using clonazepam, flunitrazepam, nitrazepam and (+)-RO-11-3128 (an active BDZ) but not (-)-RO-11-3624 (an inactive isomer). In these membranes there are high affinity and low affinity GABA binding whereas in freshly prepared membranes there are only low affinity binding sites for GABA.

Freshly prepared synaptic membranes contain an acidic protein which inhibits the Na^+-independent high affinity binding of ^{3}H-GABA. This inhibitor GABA-modulin is thermostable, has a MW of about 15,000 and has been purified 500-fold. When the membranes are frozen, thawed, repeatedly washed and then treated with Triton X-100, the GABA-modulin dissociates from the membranes. Kinetic analysis suggests that the GABA-modulin acts as an allosteric modulator for the Na^+-independent high affinity GABA binding. Diazepam (5×10^{-7}M) prevents the inhibition by this protein of ^{3}H-GABA binding to frozen and detergent-treated membranes.

These results suggest that BDZs compete with the endogenous inhibitor for the Na^+-independent high affinity binding of GABA. It has been reported that benzodiazepines work on specific "anxiety" receptors. In recent experiments we have observed that the endogenous inhibitor of GABA binding also inhibits ^{3}H-diazepam binding. One question posed by these results is whether the interaction shown in vitro in brain synaptic membranes between GABA and benzodiazepines could be just the result of homogenization or whether the three entities (GABA, benzodiazepine receptor and GABA-modulin) were part of a supramolecular organization constituting the GABA receptor-ionophore complex at the receptive site of the post-junctional neuronal membranes. To develop a simple model for studying in vivo the interaction between GABA, benzodiazepine receptor and GABA-modulin we have used different cell lines: neuroblastoma; NB2a, glioma C6, chromaffin cells from bovine adrenal and P-12 cell line.

A "GABA receptor unit" which includes brain-like ^{3}H-GABA, ^{3}H-benzodiazepine (diazepam, clonazepam, flunitrazepam) receptor binding sites and a thermostable endogenous protein (GABA-modulin) which inhibits both GABA and benzodiazepine binding has been demonstrated to be present in membranes prepared from NB2a neuroblastoma (NB) and C6 glioma (C6) clonal cell lines. In these cells, as in brain, diazepam (1 μM) prevents the effect of GABA-modulin and in turn GABA (0.1 mM) increases the binding of ^{3}H-diazepam. The NB, and to a lesser extent C6 cells, represent a suitable model to study the interactions and the sequence of membrane and intracellular events triggered by stimulation of benzodiazepine and GABA receptors. In fact in these cells muscimol and GABA increase the Cl^- influx and this increase is blocked by bicuculline. Diazepam which produces release of GABA-modulin from these cells also facilitate the increases of Cl^- flux elicited by GABA.

In conclusion, our data suggested that BDZ receptors are functionally associated with GABA receptors. Thus, the pharmacological and therapeutic action of BDZs may be mediated by an action on GABAergic neurons.

The importance of these observations may go beyond the immediate explanation of the mode of action of BDZs and may represent the beginning of a better understanding of the role of GABAergic neurons in anxiety and in other mental disorders. In addition, these observations may help in elucidating details of the supramolecular nature of postsynaptic GABA and BDZ receptors and also may offer a simple means for studying the GABAergic pathway involved in anxiety.

Publications:

Baraldi, M., Guidotti, A., Schwartz, J. P., and Costa, E.: GABA receptors in clonal cell lines: A model for study of benzodiazepine action at molecular level. Science 205: 821-823, 1979.

Costa, E.: The role of gamma-aminobutyric acid in the action of 1,4 benzodiazepines. Trends Pharmacol. Sci. 1: 41-44, 1979.

Guidotti, A., Baraldi, M., Schwartz, J. P., and Costa, E.: Molecular mechanisms regulating the interactions between the benzodiazepines and GABA receptors in the central nervous system. Pharmacol. Biochem. Behav. 10: 803-807, 1979.

Costa, E., and Guidotti, A.: Recent studies on the mechanisms whereby benzodiazepines facilitate GABAergic transmission. In Mandel, P., and DeFeudis, F.V..(Eds.): GABA-Biochemistry and CNS Function. New York, Pleum Publishing, 1979, pp. 371-378.

Guidotti, A., Baraldi, M., and Costa, E.: 1-4 Benzodiazepines and gamma-aminobutyric acid: Pharmacological and biochemical correlates. Pharmacology 19: 267-277, 1979.

Guidotti, A.: Pharmacological and biochemical evidence for an interaction between the benzodiazepine and GABA receptors. In Pepeu, G.C., Kuhar, M.J. and Enna, S.J. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1980, pp. 271-275.

Massotti, M., and Guidotti, A.: Endogenous regulators of benzodiazepine recognition sites. Life Sci., in press.

Cananzi, A. R., Costa, E., and Guidotti, A.: Potentiation by intraventricular muscimol of the anticonflict effect of benzodiazepines. Brain Res., in press.

Massotti, M., Alleva, F. R., Balasz, T., and Guidotti, A.: GABA and benzodiazepine receptors in the offspring of mothers receiving diazepam: Ontogenetic studies. Neuropharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01518-04 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The study of mammalian GABAergic mechanisms with gas chromatography-mass spectrometry and stable isotopes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. V. Revuelta	Visiting Associate	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The turnover rate of gamma-aminobutyric acid (GABA) has been measured in n. accumbens, n. caudatus, globus pallidus, substantia nigra, septum and tuberculum olfactorium where the pool of glutamate functions primarily as a GABA precursor. It has not been possible to estimate GABA turnover in areas where glutamate is a primary neurotransmitter such as deep cerebellar nuclei, cerebellar cortex, colliculus, hippocampus or in areas of the cortex (frontal, parietal, occipital or temporal). GABA turnover studies have shown that the glutamatergic collaterials from the hippocampal pyramidal cells projecting to the lateral septum do not control the GABAergic interneurons located here. However, various transmitter systems trans-synaptically modulate the GABAergic neurons located in various brain nuclei. Blockade of dopamine receptors with haloperidol increases the GABA turnover in accumbens and globus pallidus. Opiate receptor agonists and cholinergic agonists increase the GABA turnover in globus pallidus. These studies suggest that GABA turnover is a valuable tool in the study of modulation of GABA neurons by various neuronal inputs.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective has been to study the modulation of GABA neurons in various nuclei of rat brain by trans-synaptic regulation.

A recurrent problem in the study of GABA turnover rates is that glutamate functions not only as a GABA precursor but also as an intermediate metabolite and as a primary neurotransmitter. Only by selecting those tissues where the pool of glutamate that functions as a GABA precursor is rapidly formed from glucose and its rate of labelling predominates over the other two glutamate pools can the turnover of GABA be adequately estimated. In these tissues we have studied the modulation of GABA neurons by various neurotransmitter systems.

The turnover rate of GABA was measured by a sensitive gas chromatographic-mass fragmentographic technique involving infusion of ^{13}C-glucose through the tail vein of conscious immobilized animals and measurement of the relative isotopic enrichment attained by the pools of GABA and its immediate precursor, glutamic acid.

It has been possible to measure the turnover rate of GABA in nucleus accumbens, nucleus caudatus, globus pallidus, substantia nigra, septum and tuberculum olfactorium where the rate of ^{13}C-incorporation into glutamate and GABA fulfill the minimal criteria for expression as a precursor-product relationship. However, these criteria have not been fulfilled in deep cerebellar nuclei, cerebellar cortex, colliculus, hippocampus or in the areas of the cortex (frontal, parietal, occipital or temporal). Thus we have not used these areas for turnover measurements.

Bilateral transection of the cingulum (48 hr) increases the levels of glutamate (50%) and GABA (40%) in the septum but not the ^{13}C-incorporation into GABA or glutamate. Unilateral transection of the fimbria does not alter any of the GABA turnover parameters in the septum. This would suggest that the cingular pathway exerts a greater influence on the septal GABAergic interneurons than does the septal afferents coursing in the fornix/fimbria. The lack of control of the septal GABAergic interneurons by the glutamatergic collaterals of the hippocampal pyramidal cells has been implicated also by experiments involving intraventricular injection of scopolamine (10 μg; 30 min) and methacholine (10 μg; 30 min). Neither of these compounds alters the turnover of GABA in septum, accumbens or caudate. Methacholine increases the turnover rate of GABA in the globus pallidus indicating a possible cholinergic-GABAergic interaction there.

Other transmitter systems have been shown to transsynaptically modulate the GABAergic neurons located in various brain nuclei. Blockade of dopamine receptors with haloperidol appears to increase the turnover and the firing of a population of GABA interneurons in the accumbens and globus pallidus. Opiate receptor agonists increase the GABA utilization in the globus pallidus. This response appears to be associated with the catalepsy caused by opioid receptor agonists. Stimulation of opiate receptors also exerts an inhibitory influence on the turnover rate of GABA in caudate suggesting that perhaps opiate receptors are located on small GABA neurons which inhibit long axon GABA neurons of caudate. These results taken as a whole, indicate that the measurement of GABA turnover is a valuable tool in the study of the trans-synaptic modulation of GABA neurons by various neural inputs.

The study of the interactions among different neuronal systems in the brain is essential to elucidate the mechanism of action of numerous drugs. Moreover the demonstration of the intricate relationship among the neurotransmitters will contribute to a better understanding of how the diverse systems of the brain respond to drug therapy. Such knowledge would provide the background necessary to develop more effective therapeutic approaches.

Publications:

Wood, P. L., Moroni, F., Cheney, D. L., and Costa, E.: Cortical lesions modulate turnover rates of acetylcholine and γ-aminobutyric acid. Neurosci. Lett. 12: 349-354, 1979.

Peralta, E., and Costa, E.: Effect of electrical stimulation of the substantia nigra and A_{10} area on the turnover rate of GABA in n. caudatus and n. accumbens. In Usdin, E., Kopin, I. J., and Barchas, J. (Eds.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon Press, 1979, pp. 1071-1073.

Revuelta, A. V., Cheney, D. L., and Costa, E.: Measurement of γ-aminobutyric acid turnover rates in brain nuclei as an index of interactions between γ-aminobutyric acid and other transmitters. International Symposium on GABA and Glutamate as Transmitters, May 18-23, 1980, Italy, Sardinia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01519-04 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The biochemistry of cholera toxin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Trepel	Biol Lab Tech	SMRP	NIMH
	L. M. Neckers	Staff Fellow	SMRC	NIMH
Other:	D.-M. Chuang	Chemist	SMRP	NIMH
	N. H. Neff	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology and Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the biochemistry of cholera toxin and to relate the biochemistry with activation of adenylate cyclase.

PHS-6040
(Rev. 10-76)

Proposed Course:

This project has been terminated.

Publication:

Trepel, J. B., Chuang, D.-M., and Neff, N. H.: Polypeptide hormones and chromatin associated proteins act as acceptors for cholera toxin catalyzed ADP-ribosylation. J. Neurochem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01520-04 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The dynamics of catecholamine metabolism in the CNS: Biochemical pharmacology of the spinal cord catecholaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. W. Commissiong	Visiting Associate	SMRP	NIMH
Other:	F. Karoum	Staff Fellow	SMRC	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the metabolism of catecholamines in brain and spinal cord and to evaluate the factors that influence amine metabolism. Our present interest is to determine which of the coeruleus projections to the spinal cord are crossed and/or uncrossed and to evaluate the possible physiological role of this spinal projection.

PHS-6040
(Rev. 10-76)

Project Description:

We have recently provided histochemical and biochemical evidence for the existence of a major neuronal pathway projecting to the spinal cord from the locus coeruleus which contains norepinephrine. Our current objective was to determine whether the locus coeruleus neurons enter the cord crossed or uncrossed and to provide information on the physiological role of the projection.

Catecholamines and metabolites were analyzed by gas chromatography-mass spectrometry. Distribution of locus coeruleus neurons to the rat spinal cord was evaluated from the loss of norepinephrine content in the cord following lesions of the locus coeruleus. The possible physiological role of the spinal projection was assessed electromyographically in the intercollicular decerebrated rat. Based on the loss of norepinephrine content from the cord and assuming equal distribution of norepinephrine in all descending fibers, about 50 percent of the norepinephrine on each side of the cord originates from the contralateral locus coeruleus. The ipsilateral locus coeruleus apparently supplies about 30, 20 and 40 percent of norepinephrine, respectively to the ipsilateral cervical thoracic and lumbar regions of cord. Dopamine content of the cord remained unchanged after the lesion providing added support for our previous observation of an independent dopaminergic system in the cord.

Unilateral lesion of the locus coeruleus significantly reduced the norepinephrine content in the ipsilateral hippocampus but not the contralateral hippocampus. In contrast to norepinephrine, dopamine content did not change after a locus coeruleus lesion implying that there is a discrete dopaminergic innervation of the hippocampus.

Intercollicular decerebration induces muscular rigidity in the rat and the rigidity can be alleviated with the drug cyclobenzaprine. In preparations in which the locus coeruleus has been lesioned bilaterally ten days previously, cyclobenzaprine had no effect on muscular rigidity; apparently an intact coerulospinal noradrenergic projection is essential for the muscle relaxant effect of the drug. Muscle relaxation may be the consequence of activation of locus coeruleus neurons by cyclobenzaprine leading to the release of norepinephrine in the ventral horn of the cord and subsequent inhibition of motor neurons by norepinephrine.

Many of the drugs used to treat mental illness produce side effects such as abnormal motor movements as well as autonomic imbalances. Our studies are providing basic information needed to understand normal physiological spinal mechanisms in addition to the side effects of drugs. Our future studies will be directed toward evaluating the interaction of drugs with spinal catecholamine systems.

Publications:

Karoum, F., Commissiong, J. W., Neff, N. H., and Wyatt, R. J.: Biochemical evidence for uncrossed and crossed locus coeruleus projections to the spinal cord. Brain Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01521-05 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Functional role of substance P in nervous system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH
	S. E. Robinson	Staff Fellow	SMRP	NIMH
	M. Ross	Staff Fellow	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 2.4	PROFESSIONAL: 1.4	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In studies on the function of substance P and on the effect of drugs on its distribution, the possibility that a pool of substance P precursor exists must be considered. The chick embryo dorsal root ganglion contains a molecular species of high molecular weight immunoreactivity which could function as a precursor in the formation of substance P. The content of this possible precursor is regulated by treatment of ganglia with nerve growth factor. Substance P, and its apparent precursor, have also been found in superior cervical ganglia, probably located in interneurons. In order to carry out pulse chase experiments to demonstrate the nature of the relationship between this possible precursor and substance P, cultures of dissociated neurons from chick embryo dorsal root ganglia have been developed. These cultures are also useful for examining the mechanism of action of drugs such as capsaicin and for studying the question of the coexistence of two transmitters such as substance P and serotonin in the same neuron. In parallel similar studies are being carried out in the brain.

Project Description:

Evidence is accumulating that many peptides, protein hormones, and transmitters are synthesized as inactive precursors, and that the conversion of the precursor to the active component is an important step in the regulation of the modulator peptide. We have used chick embryo dorsal root ganglia, which contain substance P cell bodies, to search for a precursor to substance P. Incubation of the ganglia in vitro for 20-24 hrs resulted in an increase in the concentration of substance P; this increase was blocked by cycloheximide. In addition, incubation of 9 day chick embryo ganglia with β-nerve growth factor resulted in a further increase in the substance P content of the ganglia, an increase also prevented by cycloheximide. Gel chromatographic analysis of extracts revealed the presence of 2 peaks of substance P-like immunoreactivity (SPLI): one of which eluted at the same position as authentic substance P; the size of the other peak, of a molecular weight larger than substance P, was increased following NGF treatment. We believe this peak may be a precursor to substance P.

In order to study the relationship of the possible precursor to substance P more carefully, by means of pulse labelling and chase experiments, we have been developing a method for culture of dissociated neurons from chick embryo dorsal root ganglia. These neurons can be cultured either with serum or in a chemically defined serum-free medium, in the presence of NGF. The substance P content increases with days in culture. The cultures can be readily treated with drugs and among the studies underway is the effect of capsaicin, a possible neurotoxin which selectively reduces the substance P content. In particular, we will be interested in studying its mechanism of action. Another project will be to determine whether serotonin is present in the ganglion, whether it coexists with substance P in the same neurons, and whether serotonin-depleting drugs will also affect the substance P content.

A survey of other peripheral tissues revealed the presence of substance P in sympathetic ganglia and in submaxillary gland. The substance P content of the superior cervical ganglion was not affected by decentralization, section of the carotid sinus nerve or deafferentation, suggesting that it is present in interneurons. Chromatographic analysis revealed the presence of 2 peaks of SPLI, comparable to those in sensory ganglia. The substance P content of submaxillary gland did not arise from sympathetic innervation of the gland. However, part of it was associated with the chorda tympani and the rest appeared to be prsent in the gland cells of the submaxillary gland. Since substance P is a sialagogic compound, these results suggest that substance P in the submaxillary gland may be involved in the stimulation of salivation.

Perhaps injections of capsaicin cause specific depletion of substance P in dorsal root ganglia and spinal cord without affecting the substance P content of other peripheral tissues such as ileum and salivary gland. Peripheral capsaicin also had no effects on the substance P content of various brain regions, presumably because the neurotoxin fails to cross the blood-brain barrier. Experiments are being carried out to look at the effect of intraventricular injections of capsaicin on the substance P content of several brain regions. The content of serotonin, as well as the effect of serotonin depleters, will also be examined.

Publications:

Neckers, L. M., Wyatt, R. J., Speciale, S.G., and Schwartz, J. P.: Substance P afferents from the habenula innervate the dorsal raphe nucleus. Exp. Brain Res. 37: 619-623, 1979.

Robinson, S. E., Schwartz, J. P., and Costa, E.: Substance P in the superior cervical ganglion and the submaxillary gland of the rat. Brain Res. 182: 11-17, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01524-05 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Evidence for peripheral dopaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Z. Lackovic	Visiting Associate	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH
	J. Kleinman	Staff Psychiatrist	SMRC	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine receptors are found throughout the cardiovascular system. Their presence in peripheral structures suggests that peripheral nerves containing dopamine as a transmitter might innervate these receptors. Our objective was to systematically study the catecholamine content of peripheral nerves and organs of animals as well as human tissues taken at autopsy for the presence of dopamine and its metabolites.

PHS-6040
(Rev. 10-76)

Project Description:

There is now evidence for the presence of dopamine receptors throughout the cardiovascular and urogenital systems. These observations are consistent with the notion that there might be a peripheral dopaminergic neuronal system. Our objective was to evaluate whether peripheral nerves contain dopamine and its acidic metabolites in sufficient quantities compared with norepinephrine to suggest that there might be peripheral dopaminergic nerves.

Dopamine and its metabolites and norepinephrine were assayed in human samples obtained at autopsy and animal tissues by gas chromatography mass spectrometry (GC-MS). GC-MS is the only instrument that will provide high sensitivity together with absolute identification which is essential for this project for dopamine and related substances.

All nerves and peripheral tissues taken from man, rat and cattle contained dopamine and norepinephrine. The concentration of dopamine varied between 1.5-300 percent of norepinephrine. Thus, it appears that in some structures dopamine probably serves as a neurotransmitter rather than solely as a precursor for norepinephrine. For example, in bovine ventral and dorsal spinal nerve roots the concentration of dopamine is about three times higher than the content of norepinephrine. In man, the content of catecholamine varies depending on the origin of the nerve root. Catecholamines are higher in the roots arising in the thoracic regiona and decrease gradually until they are just detectable in the lumbar region of the cord. At thoracic 6, norepinephrine is about four times higher than dopamine but after about thoracic 8, the content of norepinephrine and dopamine are equal.

Vascular tissue contains relatively high concentrations of dopamine as well. Dopamine content in the human renal and mesenteric artery is about 15 percent of the norepinephrine content while in the femoral artery dopamine was about 4 percent of the norepinephrine content. Moreover, the tissues contained acidic metabolites of dopamine at concentrations that were about ten times the concentration found in blood. In the urogenital tissues of the rat, the dopamine content is from 1.4 to 200 percent higher than that of norepinephrine. Rat testes contained about twice as much dopamine as norepinephrine.

Our survey clearly shows that the dopamine content is rather high in many peripheral structures, that its concentration does not vary in parallel with norepineprhine and that dopamine metabolites are probably formed in peripheral structures from dopamine. These observations are consistent with the notion that there is a peripheral dopaminergic neuronal system. This system may be present to serve the many dopamine receptors that have been observed by other investigators.

Schizophrenia is associated with abnormal metabolism of dopamine and neuroleptic drugs are thought to be beneficial because they act at dopamine receptors. Our studies suggest that neuroleptic drugs might produce some of their side effects by acting at peripheral sites. Moreover, some of the symptoms of Parkinson's disease might be the consequence of abnormal metabolism of dopamine in peripheral tissue. Of greater importance, however, is the possibility of investigating a new neuronal system whose physiological function is relatively unexplored.

Following completion of our present studies we will direct our interest toward establishing the origin and physiological role of the peripheral dopamine.

Publications:

Lackovic, Z., and Neff, N. H.: Evidence for the existence of peripheral dopaminergic neurons. Brain Res., in press.

Karoum, F., Speciale, S. G., Jr., and N. H.: 4,4-dihydroxyphenylacetic acid content of sympathetic ganglia as a possible biochemical indicator of small intensely fluorescent cell participation in ganglionic transmission. Biochem. Pharmacol. 29: 118-119, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01525-04 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of nerve growth factor content of nervous system-derived tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH
	P. Onali	Visiting Fellow	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.6	PROFESSIONAL: 1.6	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nerve growth factor (NGF) is synthesized and secreted by rat C6 glioma, a nervous system-derived cell line. The NGF content of these cells is increased by β-adrenergic agonists, which cause an elevation of cyclic AMP content and the concomitant activation of cAMP-dependent protein kinase. The increase of NGF content does not depend on the increase in protein synthesis which is elicited by isoproterenol, whereas the increase of cyclic nucleotide phosphodiesterase (PDE) does. There are two forms of PDE in the cells; the cyclic AMP-specific form is induced by β-adrenergic agonists, whereas the second form hydrolysis cAMP or cGMP and is regulated by calcium and calmodulin. The induction of PDE requires translocation of the catalytic subunit of cytosol protein kinase into the nucleus, a process which is inhibited by treatment of the cells with vinblastine or colchicine. Phosphorylation of acidic nuclear proteins is also required for PDE induction; this phosphorylation can be blocked by cordycepin. RNA polymerase II is required for synthesis of PDE mRNA and treatment of the cells with either actinomycin D or α-amanitin inhibits RNA polymerase and blocks PDE induction.

PHS-6040
(Rev. 10-76)

Project Description:

The nervous system-derived cell line C6 glioma synthesizes and secretes NGF. The cells are grown in monolayer culture using standard tissue culture methods. β-NGF has been measured by two different assays: a radioimmunoassay utilizaing ^{125}I-NGF and antibody to β-NGF, and a bioassay which measures the outgrowth of neurites of dorsal root ganglia from 9-10 day chick embryos cultivated in presence of various concentrations of β-NGF. The content of -NGF as well as the amount released in the medium can be increased by treating the cells with agents which increase cyclic AMP content by adenylate cyclase activation or by inhibition of PDE. Incubation of the glioma cells with a β-adrenergic receptor agonist causes an elevation of the cellular cyclic AMP content that is maximal in 15 minutes. Cyclic AMP-dependent protein kinase is maximally activated in 30-60 mintues, and then returns to basal activity by 3 hrs. The glioma cells contain both a cyclic AMP-dependent and -independent protein kinase. The cyclic AMP-dependent form has been characterized as a Type II protein kinase on the basis of its elution pattern from a DEAE-cellulose column using a NaCl gradient.

Characterization of the time course and extent of protein kinase activation is the first step in determining how the cyclic AMP-activated protein kinase is increasing the β-NGF content of the cells. Following activation of the cyclic AMP-dependent protein kinase, there is a translocation of the catalytic subunit of the kinase from the cytosol to the nucleus. Purified nuclei from the glioma cells contain a protein kinase which is active against endogenous substrates and which could not be activated by the addition of cyclic AMP, or of calcium in the presence or absence of the calcium ionophore A23187. By 45-60 min following treatment of the cells with isoproterenol, there is a 60-300% increase of nuclear protein kinase activity. The increased activity can be inhibited by the Type I protein kinase inhibitor specific for cyclic AMP-dependent protein kinase, whereas the protein kinase activity of control nuclei cannot; this supports the concept that it is the catalytic subunit of the cyclic AMP-dependent protein kinase which is translocated into the nucleus following isoproterenol treatment. At this stage of our research we do not know whether also the regulatory subunit of the cyclic AMP-dependent protein kinase is translocated to the nucleus.

One consequence of isoproterenol activation of adenylate cyclase in the glioma C6 cells is an induction of cyclic AMP phosphodiesterase (PDE), a process which reaches a peak in 3-4 hrs and requires new protein synthesis. The cell cytoplasm contains 2 forms of PDE, which are separable on a DEAE-Sephacel column. The first form utilizes both cyclic AMP and cyclic GMP as substrates and is activated by calcium and calmodulin. The second form, which acts only on cyclic AMP, is specifically induced by isoproterenol treatment. Further characterization of the kinetics of these two forms is being carried out.

Pretreatment of glioma cells with vinblastine or colchicine blocks the increase of nuclear protein kinase and the increase of PDE activity elicited by isoproterenol. These results suggest first that the translocation of activated subunits of protein kinase from cytosol to the nucleus is required for the induction of new synthesis of PDE molecules. In addition, since vinblastine and colchicine inhibit microtubule polymerization, the results suggest that microtubules are involved in the translocation process. Regulation of protein phosphorylation depends on activation of protein kinase, location of the activated enzyme, and specific substrates present in the sites where activated enzyme is located. An yet unidentified nuclear acidic protein(s) represents a substrate for the translocated kinase. At 1-2 hrs following isoproterenol, there is increased phosphorylation of the acidic protein fraction, with no change in the degree of phosphorylation of either histones or the remainder of the nuclear proteins. Both the increased phosphorylation of acidic proteins and the PDE induction can be blocked by cordycepin, suggesting that acidic proteins regulate expression of the gene for PDE. RNA polymerase II is also required for induction of PDE. Its activity in vitro as well as the induction of PDE can be blocked by either actinomycin D or α-amanitin.

Since, neither cycloheximide nor vinblastine affect the increase in β-NGF content elicited by isoproterenol, it is inferred that at least the early (3 to 6 hr after isoproterenol) increase in NGF is not the result of new protein synthesis. We believe that this increase is the result of the activation of a process whereby pro-β-NGF is converted into NGF. We are currently in the process of isolating pro-β-NGF from mouse submaxillary gland in order to examine more closely the role that the peptidase subunit of 7s NGF plays in the conversion process and the role of the cyclic AMP-dependent protein kinase in activating this process.

Publications:

Schwartz, J. P., and Costa, E.: Protein kinase translocation following β-adrenergic receptor activation in C6 glioma cells. J. Biol. Chem. 255: 2943-2948, 1980.

Schwartz, J. P., and Costa, E.: β-Adrenergic receptor-mediated regulation of cyclic nucleotide phosphodiesterase in C6 glioma cells: Vinblastine blockade of isoproterenol induction. J. Pharmacol. Exp. Ther. 212: 569-572, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01526-04 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Modulation of tyrosine hydroxylase activity in retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cohen	Staff Fellow	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms that are responsible for modulating tyrosine hydroxylase activity in retina. This enzyme is apparently rate limiting for catecholamine biosynthesis. Our present interest is to determine whether tyrosine hydroxylase activity of retina can be activated by neuroleptic drugs similar to the enzyme associated with the nigrostriatal system of brain.

Project Description:

Dopamine, a putative neurotransmitter in the central nervous system of mammals, is contained in a subpopulation of retinal amacrine neurons. Exposure to light activates retinal tyrosine hydroxylase activity (concomitant with an increase of dopamine synthesis (see Z01 MH 01526-02,03). Activation of the enzyme is characterized by a decrease of the Km for the pteridine cofactor. Our objective was to determine whether neuroleptic drugs that activate tyrosine hydroxylase of the nigrostriatal system of brain would activate the enzyme of retina.

Rats were placed in a light controlled environment with lights on for 12 hrs. Drugs were administered on the eleventh hour of darkness and the animals were kept in the dark following treatment. Enzyme activity and kinetics were evaluated by standard procedures. Haloperidol, chlorpromazine, clozapine, or domperidone treatment resulted in activation of tyrosine hydroxylase in the dark. Activation was dose-related. With haloperidol, enzyme activation was maximal within 30 minutes and activation persisted for more than three hours. By kinetic analysis, activation was characterized as a decrease of the Km for the pteridine cofactor similar to the kinetic changes observed when animals are exposed to light.

Neuroleptic drugs excite the nigrostriatal system of brain apparently by activation of a neuronal feedback system. Our results suggest that the state of retinal tyrosine hydroxylase activity is modulated, in part, by a neuronal feedback loop system as well. The retinal dopaminergic system offers a simple model for evaluating the biochemical processes that are responsible for the physiological and pharmacological activation of tyrosine hydroxylase. Moreover, the system may give insight into pigmentary retinopathy which has been reported in man following treatment with neuroleptic drugs.

Our future studies will be directed toward preparing isolated amacrine cells to enable us to study the cell surface receptors that participate in amacrine cell activation.

Publication:

Iuvone, P. M., Joh, T. H., and Neff, N. H.: Regulation of retinal tyrosine hydroxylase: Long term exposure to light increased the apparent Vmax without a concomitant increase of immunotitratable enzyme molecules. Brain Res. 178: 191-195, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01527-04 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of endogenous opioids using high pressure liquid chromatography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Group on High Pressure Liquid Chromatography

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Separation of many peptides present in tissue which often are structurally very similar requires the highest possible resolution. High pressure liquid chromatography (HPLC) probably has the highest resolution for peptides of any method available today, but incomplete understanding of parameters affecting resolution and retention has hindered the application of the method. A number of factors controlling retention of peptides in HPLC were evaluated in this study. These results will aid in rapid specific isolation of peptides for quantitative analysis.

PHS-6040
(Rev. 10-76)

Project Description:

High pressure liquid chromatography (HPLC) is now being used in many labs for isolating and purifying small peptides. Although electrophoresis permits higher resolution of proteins and large peptides (>50 amino acid residues) HPLC provides for greater resolving power for separation of small peptides than any other available method. The development of HPLC methods for opioid peptides or other neuropeptides has been largely a matter of trial and error because of the lack of understanding of basic principles that regulate the chromatographic separation of peptides and the similarity and number of different peptides present in tissues. The highest possible resolving power is needed for peptide separation. Ideally, HPLC should separate a desired peptide from all other compounds, including closely related deletion or addition of homologs. Typically, HPLC is used for peptide isolation prior to amino acid analysis, sequencing determination of catabolism and verification of radioimmunoassay. The chromatographic step must therefore be able to separate peptides differing by only a single amino acid residue to avoid equivocal results in the experiment. In order to utilize the full potential power of HPLC, one needs to understand what are the factors that govern the retention of these complex compounds by the HPLC column. Furthermore, the many factors that affect resolution (such as mobile phase composition, flow rate, gradient shape, antitailing additives) must be better understood in order to maximize the resolution of HPLC.

The objectives of this project are to determine what controls retention of peptides on HPLC columns and what conditions are necessary for maximum resolution.

The methodology employed involves reverse phase separation of synthetic peptides with detection by measurement of UV absorption (210 nm). Computers are used for on line control of the HPLC system and data acquisition and off line for data transformation and analysis.

Major Findings

1) Retention of peptides in reverse phase HPLC depends almost entirely on the amino acid composition of the peptide.

2) The peptides' conformation, sequence or terminal residue have almost no effect on retention.

3) A nomogram was developed which describes the relationship of the percentage of the organic solvent in the mobile phase vs the HPLC retention for a wide variety of compounds.

4) This nomogram will be of value for optimization of mobile phases to any worker employing reverse phase HPLC.

5) An inexpensive computer based analysis system was developed to determine optimum analysis conditions by precise evaluation of chromatographic parameters (peak variance, skew peak resolution, peak spacing). Experiments are now underway utilizing this system.

The significance of these findings lies in the growing importance of neuropeptides in regulation of brain function, and in the great need of methods for isolation and purification of these peptides for measurement or subsequent analysis. Work from this laboratory has contributed to our knowledge of the rules that regulate peptide retention time. The needed systematic studies of factors affecting resolution are now underway.

Publications:

Meek, J. L.: Prediction of peptide retention times in high pressure liquid chromatography on the basis of amino acid composition. Proc. Natl. Acad. Sci. USA 77: 1632-1636, 1980.

Meek, J. L.: Nomogram for adjusting mobile phase composition in reverse phase high pressure liquid chromatography. Anal. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01530-04 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regional distribution and regulation of enkephalin in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.S. Hong	Staff Fellow	SMRP	NIMH
Other	H.-Y.T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hippocampus participates in the regulation of emotions and contains small amounts of met^5-enkephalin but a relatively high amount of receptors. The met-enkephalin content differs in various regions of the hippocampus. The temporal region contains three times more met-enkephalin than the septal region. The CA3 and CA4 regions contain twice the met-enkephalin content of CA1, CA2, and subiculum. The uneven distribution of met-enkephalin within the hippocampus suggests that met-enkephalin neurons may be located in specific sites where they regulate pyramidal cell activity. Studies using both mechanical and chemical lesions suggest that ME containing neurons are short axon interneurons within the hippocampus. The hippocampal ME content increases after the animals exhibit recurrent motor seizures. This result suggests that the met-enkephalin neurons may respond to certain pathological conditions and may be associated with the postictal depression.

PHS-6040
(Rev. 10-76)

Project Description:

Evidence has suggested that opiates or endorphins exert a powerful influence on the hippocampal formation. Intraventricular injections of beta-endorphin trigger recurrent changes in the hippocampal EEG which are characterized by high voltage activity and convulsive-like discharges. Microinjection of Leu^5-enkephalin (LE) into the dorsal hippocampus of cats and rats produced localized epileptiform discharges of electrical activity; this epileptogenic action of LE is prevented by a microinjection of naloxone in the same site. Administration of opioid peptides by intraventricular injection or by iontophoresis onto the cells increases the activity of the hippocampal pyramidal cells presumably by inhibition of neighboring basket cells. The purpose of this project was to examine the distribution of met^5-enkephalin (ME) and location of ME containing neurons within the hippocampus. In attempting to localize the cell bodies of ME-containing neurons which project their axons to the hippocampus, both mechanical and chemical lesions were performed. The results suggest that ME-containing neurons are intrinsic to the hippocampus. While performing kainic acid lesions we observed that recurrent convulsions can change the ME content of hippocampus. This prompted us to study the effects of convulsions on hippocampal ME.

To characterize the chemical nature of the ME-like material measured by radioimmunoassay (RIA), hippocampal extracts were treated with pronase and RIA was performed before and after pronase treatment. Pronase treatment destroyed the immunoreactivity of hippocampal extracts suggesting that the material measured is a peptide. In addition, the extracts were chromatographed with Biogel P-2 columns and the immunoreactivity was determined in each fraction of the eluate. The chromatographic profile shows that one peak of immunoreactivity was detected; this peak was eluted at the same position as authentic ME.

ME content differs in various regions of the hippocampus. The temporal region contains three times as much ME as the septal region. The CA3 and CA4 regions contain twice as much ME as CA1, CA2 and subiculum.

Hippocampal ME content increases by 2 to 3-fold after rats exhibit recurrent motor seizures induced by intracerebral injections of kainic and repeated electroconvulsive shock or systemic injections of isoniazid in doses which cause recurrent convulsions. The aforementioned increase in ME content can be prevented by pretreatment with anticonvulsants such as phenobarbital, and muscimol. This study demonstrates that the metabolic state of ME stored in hippocampal neurons can change in response to recurrent seizures suggesting that enkephalin may play an important role in regulating the hippocampal activity.

Publications:

Hong, J.S., Wood, P. L., Gillin, J.C., Yang, H.-Y.T. and Costa, E.: Changes of hippocampal Met^5-enkephalin content after recurrent motor seizures. Nature 285: 231-232, 1980

Hong, J. S., Wood, P. L., Gillin, J. C., Yang, H.-Y.T. and Costa, E.: Recurrent convulsions and hippocampal met^5-enkephalin content. In: E. Costa and M. Trabucchi (eds.): Advances in Biochemical Psychopharmacology, Vol. 22, New York, Raven Press, pp 385-398, 1980

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01531-03 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Nerve growth factor in human fibroblasts from controls and familial dysautonomia patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH

COOPERATING UNITS (if any)

Department of Human Genetics, Yale University Medical School,
New Haven, Connecticut

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Skin fibroblasts from human controls or from patients with familial dysautonomia (FD) have been grown in tissue culture. β-Nerve growth factor (NGF) has been measured in extracts from both type of cells, using a radioimmunoassay and a bioassay. The FD cells have contents of NGF as measured by RIA comparable to the controls. However, the biological activity of the FD samples is about one-tenth that of the controls when expressed as biological units of activity per ng β-NGF as measured in the RIA. Stimulation of β-adrenergic receptors in both cell lines with l-isoproterenol results in a 17-170 fold rise in intracellular cyclic AMP in 10 min. The NGF content of the control fibroblasts increased 50-300% after 3-4 hrs of exposure to isoproterenol whereas there was no change in the NGF content of the FD cells at any time.

Project Description:

Familial dysautonomia (Riley-Day Syndrome) is an autosomal recessive disease, in which patients have impaired autonomic and sensory functions. Symptomatically, these patients resemble animals that have been treated with antiserum to NGF. It has been suggested that there may be an impairment of NGF associated with the disease, and there are reports that FD patients have elevated serum NGF levels.

We have chosen to use skin fibroblasts from FD patients, and fibroblasts from paired controls, to study this problem further. The fibroblasts are grown in monolayer culture, using early passage cells and standard tissue culture procedures. NGF is assayed using a radioimmunoassay which detects β-NGF as well as the β-component of 7s NGF, and also a bioassay in which neurite sprouting from 9 day chick embryo dorsal root ganglia is measured.

NGF remains essentially constant in both control and FD fibroblasts as they go from log to stationary phase growth. Control and FD cells have been found to contain comparable amounts of NGF as measured by radioimmunoassay. However, estimation of the NGF content of the same extracts by a bioassay procedure shows that the extracts from FD cells are much less biologically active than cells from paired controls. When expressed as biological units of NGF per ng β-NGF (as measured in RIA), there is a ten-fold difference in biological activity. In contrast, fibroblasts from patients with dystonia musculoram deformans, another genetically inherited neurological disease, contained levels of biological activity per ng of immunoreactive β-NGF that are similar to those of control extracts. Three dystonia lines, five FD lines and seven control lines have been assayed.

These fibroblasts have a β-adrenergic receptor linked to adenylate cyclase. Stimulation with l-isoproterenol caused a 17- to 170-fold increase of cyclic AMP in all lines tested. In the control lines, NGF content increased 50-300% within 3-4 hrs following isoproterenol. However there was no change in the NGF content of any FD cell line over the course of a 7.5 hr treatment with isoproterenol. These results suggest that there may be a defect in the processing of a pro-β-NGF in familial dysautonomia.

The defect could lie in the pro-β-molecule, in the converting peptidase, thought to be the γ-subunit of 7s NGF, or at some other step. Research is now being carried on to characterize the chemical differences between the NGF of control and FD fibroblasts. One approach has been to develop an isolation procedure for the pro-β-NGF molecule, in order to prepare antibodies against it and thereby develop an assay, as well as to do chemical studies on its structure, for comparison ultimately with the structure of the pro-β-NGF from FD extracts. Submaxillary glands removed from castrated male mice which were treated with testosterone provide a relatively enriched source of the pro-β-NGF. Chromatography of an extract of these glands over a Sephadex G-100 column resulted in fractionation of β-NGF immunoreactivity into three peaks, the first of which is the large MW 7s NGF. Currently we are characterizing the other two peaks to determine if incubation with the γ-peptidase could convert either of them into β-NGF. The alternative approach has been to incubate the fibroblast cultures with radioactive amino acids, carry out an immunoprecipitation on the cell extracts with the β-NGF antiserum and separate the immunoprecipitated material on some type of polyacrylamide gel.

Because of the increasing use of amniocentesis for prenatal diagnosis, we have been studying the feasibility of growing fibroblasts from the amniotic fluid and measuring the NGF content by both radioimmunoassay and bioassay. Preliminary results suggest that this approach may provide a method for prenatal diagnosis of familial dysautonomia.

Publications:

Schwartz, J.P., and Breakefield, X.O.: Altered nerve growth factor in fibroblasts from patients with familial dysautonomia. Proc. Natl. Acad. Sci. USA 77: 1154-1158, 1980.

Breakefield, X.O., Edelstein, S.B., Grossman, M.H., and Schwartz, J.P.: Variations in MAO and NGF in cultured human skin fibroblasts. In Gershon, G.S., Matthysse, S., Cianarello, R.D. and Breakefield, X.O. (Eds.): Genetic Strategies in Psychobiology and Psychiatry. Calif., Boxwood Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01532-03 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of catecholamine receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Chuang	Chemist	SMRP	NIMH
Other:	W. Kinnier	Staff Fellow	SMRP	NIMH
	L. Farber	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This loss of membrane-bound beta-adrenergic receptor recognition sites in frog erythrocytes during subsensitivity induced by exposure of cells to isoproterenol is due at least in part to the internalization of beta-receptor recognition sites. When the beta-adrenergic receptors are resensitized, the amounts of internalized receptor recognition sites are also returning to the normal. The internalized recognition sites of beta-adrenergic receptors have properties identical to those of membrane-bound receptors. Glycoprotein seems to play an important role in triggering this event. A series of tricyclic antidepressant drugs was found to inhibit the receptor internalization; these effects can be attributed to an inhibition of these drugs of the agonist binding to beta-receptor recognition sites in erythrocyte membranes. Inhibitors of transglutaminase diminish the extent of recognition site internalization with a concomitant prevention of the loss of membrane-bound receptors elicited by isoproterenol. There is an excellent correlation between the inhibition of recognition site internalization and the potency of these compounds to block the transglutaminase activity in vitro.

PHS-6040
(Rev. 10-76)

Project Description:

We have continued our study on the internalization of the recognition sites of beta-adrenergic receptors in frog erythrocytes during beta-adrenergic receptor desensitization produced by incubation with isoproterenol. The extent of this internalization is measured by the increase in the number of ^{3}H dihydroalprenolol binding sites present in the cytosol of erythrocyte homogenate. The time course of internalization coincides with that of the decrease in the density of membrane-bound beta-adrenergic receptors. When the receptors are resensitized by bringing the red cells into an agonist-free medium, the amounts of internalized receptors revert toward the control value. Concomitantly the number of membrane-bound beta-receptor and the responsiveness of adenylate cyclase to isoproterenol stimulation are restored to the normal.

The internalized recognition sites appear to have a specificity for beta-adrenergic receptor agonists similar to that of membrane-bound recognition sites; neither the catalytic subunits of adenylate cyclase nor the GTP binding sites were found to be internalized during beta-adrenergic receptor subsensitivity. This receptor internalization is temperature-dependent and requires metabolic energy. Preincubation of cells with doses of concanavalin A which induce erythrocyte agglutination was found to inhibit the internalization of beta-receptor recognition sites, suggesting that glycoproteins play a role in causing this event. Preincubation of red cells with micromolar concentrations of tricyclic antidepressant drugs (such as imipramine, desipramine or iprindol) also diminishes the extent of recognition site internalization elicited by isoproterenol. This event can be attributed to an inhibition of the agonist binding to the surface receptors of erythrocytes. These results may suggest the existence of the interaction between beta-receptors and tricyclic antidepressant binding sites in post-synaptic cells.

Transglutaminase, a Ca^{++}-dependent cross-linking enzyme, has been shown to be essential for the internalization of polypeptides and their receptors following the binding of the polypeptide ligands to the plasma membranes in several systems. This enzyme appears to mediate the clustering of polypeptide molecules over coated pits of plasma membranes; this receptor clustering is followed by the invagination of coated pits to form coated vesicles and the translocation of these vesicles to organelles. We have therefore examined whether similar mechanisms are operating during the internalization of beta-receptor recognition sites of frog erythrocytes. Seven inhibitors of transglutaminase were found to inhibit the internalization of beta-receptors with a concomitant prevention of the loss of membrane-bound receptors occurring during isoproterenol-elicited subsensitivity. Moreover in a series of molecules there is an excellent correlation between the ranking of their potency which inhibit internalization and desensitization and the potency of these compounds to block the transglutaminase activity in vitro. These results have provided further evidence for the similarity in the mechanisms whereby beta-receptor and polypeptide receptors are internalized. Experiments employing fluorescent microscopy to demonstrate directly the clustering and internalization of beta-receptors are now in progress.

Publications:

Chuang, D. M., Farber, L., Kinnier, W. J., and Costa, E.: Beta-adrenergic receptors from frog erythrocytes: Receptor internalization as a mechanism for receptor desensitization. In Pepeu, G., Kuhar, M. J. and Enna, S. J. (Eds.): Receptor for Neurotransmitters and Peptide Hormones. New York, Raven Press, in press.

Chuang, D. M., Kinnier, W. J., Farber, L., and Costa, E.: A biochemical study of receptor internalization during beta-adrenergic receptor desensitization in frog erythrocytes. Mol. Pharmacol., in press.

Chuang, D. M., and Costa, E.: Involvement of transglutaminase in the internalization of beta-adrenergic receptors in frog erythrocytes. Submitted to Nature.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01533-03 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Regulation of tissue stores of beta-endorphin-like immunoreactivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	S.M. Govoni	Guest Worker	SMRP
Other:	H.-Y.T. Yang	Pharmacology	SMRP
	E. Costa	Chief	SMRP

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Section on Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to understand the involvement of beta-endorphin in the development of obesity, beta-endorphin immunoreactivity was studied in genetically obese male and female mice (C57Bl/SJ ob/ob) and their lean littermate controls (+/?). At three month's of age, the beta-endorphin immunoreactivity in pituitary glands of female obese mice is significantly greater than that of female lean controls. No such difference was observed in male obese and control mice. Since both sexes at three months of age have displayed obvious obesity, the results suggest that increased beta-endorphin may not be directly involved in the development of The results further suggest that beta-endorphin synthesis in obese mice may be modulated by sex hormones.

PHS-6040
(Rev. 10-76)

Project Description:

Several lines of evidence suggest that beta-endorphin, its precursors, and its metabolites may be involved in the development of obesity. For example, induction of compulsive eating behavior by hypothalamic injection of beta-endorphin, suppression of food intake by naloxone administration, and increased content of pituitary beta-endorphin-like material in genetically obese mice have been observed by various investigators. In order to understand whether the metabolism of beta-endorphin is changed in the pituitary and hypothalamus of obese mice,the hypothalamic and pituitary contents of beta-endorphin and its precursors, proopiocortin and beta-lipotropin were studied in genetically obese male and female mice (C57BL/SJ ob/ob) and their lean litter mates (+/? control). In three month old mice, the total pituitary beta-endorphin immunoreactivity of female obese mice is significantly greater than that of female lean mice; while, in ob/ob and +/? male mice of the same age, no difference was observed. Further analysis of the immunoreactive material revealed that the increased beta-endorphin immunoreactivity is due mainly to an increase in the beta-endorphin content; the beta-lipotropin content appears to be normal. No changes were observed in the beta-endorphin immunoreactivity in hypothalami of ob/ob mice and +/? controls of both sexes. These results clearly suggest that 1) the increase of pitiutary beta-endorphin immunoreactivity may not be directly related to obesity, since three month old mice of both sexes have shown obvious obesity; 2) the metabolism of beta-endorphin in obese mice may be modulated by sex hormones, and 3) the obese mice may be an interesting model to study the role of beta-endorphin in peripheral endocrine function.

We are currently studying the effect of estrus cycle on the beta-endorphin secretion from pituitary.

Publications:

Fratta, W., Yang, H.-Y.T., Majane, E., and Costa, E.: Distribution of beta-endorphin and related peptides in the hypothalamus and pituitary. Neuroscience 4: 1903-1908, 1979

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01534-03 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Location and role of met^5- and leu^5-enkephalin and enkephalin-like peptides in neuronal tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
Other:	E. Majane	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Enkephalin-like material in adrenal gland consists of high and low molecular weight forms. The high molecular weight (HMW) forms (MW >1000) are N-terminal extended met^5-enkephalins and show no opiate-like activity unless trypsinized. They are preferentially located in chromaffin cells and seem to exist also in blood suggesting that they may be released into blood from chromaffin cells. In the plasma, the HMW forms were found to be very stable. The low molecular weight (LMW) fraction also consists of multiple forms including met^5- and leu^5-enkephalins and three other enkephalin-like peptides. They bind to opiate receptors with high affinity suggesting that they may function as opiate ligands in adrenal glands; they can be detected in splanchnic nerves and also in adrenal medulla. In view of the existence of opiate receptors on chromaffin cells and the presence of opiate peptides in the splanchnic, LMW enkephalin-like peptides may exert their action on chromaffin cells.

PHS-6040
(Rev. 10-76)

Project Description:

Two HMW forms of enkephalin-like peptides (MW 8000 and 3000, respectively) lose their immunoreactivity very rapidly when treated with carboxypeptidase A but not with leu-aminopeptidase suggesting that they contain a met^5-enkephalin sequence at their carboxy-terminals. The HMW forms are very stable in the plasma and appear to exist in blood. The chromaffin cells isolated from bovine adrenal glands contain predominately these two forms. Therefore, it is possible that the HMW enkephalin-like peptides in chromaffin cells may be secreted in blood.

The analysis of the low molecular weight fraction (MW <1000) reveals that it is composed of several immunoreactive peptides which include met^5- and leu^5-enkephalins and two or three other enkephalin-like peptides. They can also be detected by radioreceptor binding assay indicating that they bind to opiate receptors with high affinity and thus may function as endogenous opiate ligands in adrenal gland and in other peripheral tissues (blood vessels?). One of these LMW enkephalin-like peptides was found to behave very differently from met^5-enkephalin in the radioreceptor binding assay. It can be most readily detected by radioreceptor binding assay using etorphin as the radioactive ligand. Furthermore, this newly detected enkephalin-like peptide seems to account for a large percent of the opiate activity in adrenal medullary extracts using opiate receptor binding assay. LMW enkephalin-like peptides are present in splanchnic nerves and also in adrenal medulla. Since only very minute amounts of LMW enkephalin-like peptide are present in chromaffin cells. In view of the existence of opiate receptors on chromaffin cells.

The existence of heterogeneity of enkephalin-like material in bovine adrenal glands has been previously demonstrated using gel filtration chromatography in combination with radioimmunoassay. The enkephalin-like immunoreactive peptides detected in adrenal medullary extracts vary widely in their molecular weight ranging from 8000 to 600. That the detection of high molecular weight forms is not due to aggregation of the low molecular weight forms or the absorption of the low molecular weight forms into large proteins or peptides has been established by immunoabsorption experiment and gel filtration chromatography with 6M guanidine. In order to explore the role of this highly complicated system of enkephalin-like peptide in adrenal gland, a study was initiated to investigate these peptides with regard to their 1) cellular location, 2) biochemical characteristics and 3) biological properties.

A very large proportion of the total enkephalin-like immunoreactivity in bovine adrenal glands is represented by multiple forms of high molecular weight (HMW) peptides. Two of the main HMW forms have been purified and estimated to have molecular weights of around 2500-3000 and 8000. They show opiate activity in the bioassay system employing the longitudinal myenteric plexus of guinea pig ileum only after having been predigested with trypsin. Low molecular weight enkephalin-like peptides are released after trypsinization and one of them has been identified to be met^5-enkephalin. LMW enkephalin-like peptides in splanchnic nerve may exert their action on chromaffin cells.

We are planning to further characterize the various forms of enkephalin-like peptides and search for their target tissue in the periphery.

Publication:

Yang, H.-Y. T., Di Giulio, A. M., Fratta, W., Hong, J. S., Majane, E. A., and Costa, E.: Enkephalin in bovine adrenal gland: Multiple forms of met^5-enkephalin in immunoreactive peptides. Neuropharmacology 19: 209-215, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01535-03 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Quantitative multiple ion detection mass spectrometry of neuronal peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Peralta	Guest Worker	SMRP	NIMH
Other:	J. S. Hong	Staff Fellow	SMRP	NIMH
	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In search for a more specific method for met^5-enkephalin assay, a procedure based on fragmentation of the enkephalin by dipeptidyl aminopeptidase and subsequent analysis of the resultant dipeptides and amino acid was explored. In this method, met^5-enkephalin can be specifically measured without interference from leu^5-enkephalin. The method is easily applicable to the tissue in which there is a very low degree of molecular heterogeneity such as brain tissue. However, the method may be applicable to tissue, where there is a high degree of molecular heterogeneity, only after extensive prepurification.

Project Description:

It has been well recognized that met^5-enkephalin-like immunoreactive material exists in multiple forms and this molecular heterogeneity is especially pronounced in adrenal glands. Most importantly, these multiple forms differ in their biological properties. Thus it is obvious that a more specific method other than radioimmunoassay is preferred for studying the highly complexed met^5-enkephalin system in adrenal glands or other peripheral tissues. In this study, we have considered the enzymatic fragmentation of the met^5-enkephalin into characteristic dipeptides by dipeptidyl aminopeptidase I (DAPI) and the subsequent analysis of the dipeptides generated by GC-MS.

Purified DAPI was used to hydrolyze met^5-enkephalin into the dipeptides Tyr-Gly and Gly-Phe and methionine. The dipeptides could be derivatized and resolved by gas chromatography; quantitation of these dipeptides was obtained by single ion monitoring. The tissue samples were pre-purified by Bio-Beads SM-2 and then subjected to DAPI digestion. The yield of methionine and of the two dipeptides increases with time. The same dipeptides are produced from met^5-enkephalin and leu^5-enkephalin; however the ratios of Tyr-Gly/Gly/Phe and Tyr/Gly/Met may be conveniently used for the qualitative identification of met^5-enkephalin. Since the only internal standard available was deuterated methionine, quantitation of met^5-enkephalin in tissue, which exhibits a very low degree of molecular heterogeneity, was carried out by measuring the methionine released by DAPI. The present GC-MS method can only be used to quantify met^5-enkephalin in brain but not in adrenal without extensive prepurification.

This project has been terminated.

Publication:

Peralta, E., Yang, H.-Y. T., Hong, J., and Costa, E.: Combination of enzymatic and mass fragmentographic assays for the identification and measurement of met^5-enkephalin. J. Chromatogr. 190: 43-51, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01536-02 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Characterization of receptors for putative neurotransmitters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. O. Kayaalp	Visiting Scientist	SMRP	NIMH
Other:	M. Parenti	Visiting Associate	SMRP	NIMH
	S. Gentleman	Pharmacologist	SMRP	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.4	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the receptors for putative transmitter substances. Our present objective is to characterize dopamine receptor sites in brain using radioligands and to evaluate the factors that modulate dopamine activated adenylate cyclase activity.

Project Description:

Abnormal brain dopamine metabolism apparently plays a major role in the etiology of Parkinson's disease and some forms of mental disease. Our objectives were two-fold: 1) to study the binding sites for dopamine in brain tissue, and 2) to provide new information on the factors that modulate dopamine activated adenylate cyclase.

Dopamine binding sites were studied using high specific activity radiolabeled agonists and antagonists. Receptor responses were evaluated from the activation of adenylate cyclase by dopamine. To our surprise, ascorbic acid, which is usually included in the incubation mixture when studying dopamine receptor binding sites, inhibits the specific binding of the agonists, ADTN and apomorphine, but not the antagonists, haloperidol and spiroperidol. Other reducing agents had a similar action. The observation is consistent with the notion that dopamine antagonist and agonist sites are separate and distinct. Moreover, they suggest the possibility that the number of agonist sites in vivo may be modulated, in part, by the state of oxidation of the binding sites.

Recently, we observed dopamine agonist and antagonist binding sites in rat spinal cord, thus supporting our thesis (Z01 MH 01520-02) that dopaminergic neurons play a role in spinal cord physiology.

Several studies have demonstrated that opiates interfere with dopaminergic neurons in the striatum and induce pharmacological effects similar to neuroleptic drugs. We found that opiates and opioid peptides inhibit dopamine activated adenylate cyclase. Inhibition of dopamine-sensitive adenylate cyclase was stereospecific and reversible by naloxone; thus, the opiate inhibition of dopamine-activated adenylate cyclase has definitive characteristics of the opiate receptor. These observations are consistent with the view that the neuroleptic-like opiate effects are the result of reduced responsiveness of this dopamine adenylate cyclase activity following treatment with opiates. Schizophrenia has been postulated to be the consequence of excessive dopaminergic influence on brain function. Apparently Parkinsonism is the consequence of a loss of dopaminergic neurons from the nigrostriatal system. Our current studies are providing basic information needed to understand normal physiological mechanisms in addition to the side effect of drugs used to treat these diseases. Future studies will be directed towards understanding the biochemical mechanisms responsible for the blockade of dopamine activated adenylate cyclase by opiate drugs. Other endogenous peptide substances will be evaluated for their potential to inhibit adenylate cyclase systems.

Publications:

Kayaalp, S. O., and Neff, N. H.: Differentiation by ascorbic acid of dopamine agonist and antagonist binding sites in striatum. Life Sci. 26: 1837-1842, 1980.

Neff, N. H., Gentleman, S., Parenti, M., and Commissiong, J. W.: Dopamine neurons of spinal cord: A possible site for the action of neuroleptic drugs: Enzymes and neurotransmitters in mental diseases. In Usdin, E. (Ed.):

Enzymes and Neurotransmitters in Mental Disease. John Wiley and Sons, Inc., in press.

Kayaalp, S. O., and Neff, N. H.: Cholinergic muscarinic receptors of bovine adrenal medulla. Neuropharmacology 18: 909-912, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01537-02 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical pharmacology of GABA receptor system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Mazzari	Guest Worker	SMRP	NIMH
Other:	A. Leon	Guest Worker	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

M. Massotti, FDA, Bureau of Drugs, 200 C Street, Washington, D.C. 20032

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

GABA-modulin, an endogenous membrane protein, which inhibits non competitively ^{3}H-GABA binding to synaptic plasma membranes, has been isolated and characterized. This protein material is also a potent inhibitor of membrane protein phosphorylation and acts as a competitive inhibitor of the protein kinase substrates. The role of GABA-modulin in the control of GABA receptor system function is presently being investigated.

Project Description:

When GABA receptor sites are occupied by the endogenous agonist the membrane permeability to Cl$^-$ ion changes, resulting in a hyperpolarization or depolarization of the receptive neurons. The goal of this project is to provide a better understanding of the function of the GABA receptor complex and in particular the coupling of GABA recognition site with the Cl$^-$ ionophore as a first step in developing new potent and specific drugs which like benzodiazepines can alter the function of the GABA receptors.

Our studies indicate that in freshly prepared crude synaptic membranes, purified synaptic plasma membranes, or membranes from neuroblastoma clonal cell lines, contain only one population of ^{3}H-GABA binding sites. Repeated washings of these membranes combined with freezing, thawing and treatment with Triton X-100 unmasks an additional population of GABA binding sites characterized by high affinity for the agonist. Subjecting the Scatchard plot of these binding studies to the graphic analysis of Rosenthal for one ligand and two types of binding sites, the total density of GABA receptor sites and the relative portion of high (Kd 20-40 nM) and low affinity component (Kd 200-400 nM) of ^{3}H-GABA binding can be estimated.

The increase in number of high affinity binding sites for GABA after Triton X-100 treatment is most likely due to the removal of an endogenous inhibitor protein (GABA-modulin) from the membranes because the increase of ^{3}H-GABA binding is paralleled by a decrease of GABA-modulin content in these membranes. Furthermore the addition of small amounts of purified GABA-modulin to the Triton X-100 treated membranes readily masks the population of receptor with high affinity binding sites.

Using filtration procedures on Biogel P-2 column and Sephadex G-50 column, GABA-modulin has been extracted and purified from synaptic plasma membranes or from fresh or microwaved brain. It presents the following characteristics: 1) it is thermolabile; 2) it inhibits high affinity ^{3}H-GABA binding non competitively; 3) prevents GABA stimulated ^{3}H-diazepam binding; 4) is trypsin and pronase sensitive; and 5) is not dialyzable. In addition it contains inhibitory activity for GABA binding and for protein phosphorylation. These data suggest that the inhibitor of GABA binding is identical in nature with the Type II inhibitor of protein kinases previously described and characterized.

In agreement with this observation removal of GABA-modulin from synaptic membranes, using Triton X-100, resulted in an increase of membrane phosphorylation. The extent of this increase is similar to the increase in GABA binding observed in the same preparation. Both the increase in GABA binding and the increase in protein phosphorylation are nullified by the addition of purified GABA-modulin. These experiments indicate that GABA-modulin may interfere with the function of the GABA receptor unit perhaps by altering the state of membrane phosphorylation.

Evidence has been accumulated to suggest that GABAergic abnormalities may be related to the symptoms of Huntington's disease, Parkinson, epilepsy and possibly schizophrenia. Thus biochemical and pharmacological characterization of the GABA receptor system may be a relevant approach to find compounds which are capable of modifying the GABAergic system in man.

In future experiments we plan to develop a simple method to measure GABA-modulin content in different biological samples and to understand whether drugs that interfere with GABA-modulin action may be useful in the control of the GABA receptor system in neurological and/or psychiatric disorders.

Publications:

Guidotti, A., Gale, K., Suria, A., and Toffano, G.: Biochemical evidence for two classes of GABA receptors in rat brain. Brain Res. 172: 566-571, 1979.

Toffano, G., Leon, A., Massotti, M., Guidotti, A., and Costa, E.: GABA-modulin: A regulatory protein for GABA receptors. In Kuhar, M.J., Enna, S.J. and Pepeu, G.C. (Eds.): Receptors for Neurotransmitters and Peptide Hormones. New York, Raven Press, pp. 133-142, 1980.

Costa, E., Guidotti, A., Massotti, M., and Mazzari, S.: The regulation of GABA receptors as a possible mechanism for GABA 1,4-benzodiazepine interactions. EMBO Workshop on Drug Receptors in the Central Nervous System, held in Jerusalem, Israel.

Mazzari, S., Leon, A., Massotti, M., Guidotti, A., and Costa, E.: Assaying GABA-modulin activity in brain extracts: Resolution of inherent difficulties. Proc. Conference on Psychopharmacology and Biochemistry of Neurotransmitter Receptors, held in Tucson, Arizona.

Mazzari, S., Massotti, M., Guidotti, A., and Costa, E.: GABA receptors as supramolecular units. International Symposium on GABA and Glutamate as Transmitters, held in Sardinia, Italy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01538-02 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The biochemistry of hippocampal synapses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Kinnier	Staff Fellow	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
Mr. P. Oliver, Office of the Division Chief
Dr. D.L. Cheney, Chief of the Section of Molecular Pharmacodynamics, L.P.P.

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regional distribution of acetylcholine (ACh) and cyclic GMP, ACh turnover rates, ACh fractional rate constant, affinity and number of binding sites for $[^3H]$quinuclidinyl benzylate (QNB) were measured in various regions of the rat hippocampus. In coronal sections of the hippocampus, both ACh and cyclic GMP content were found to be the highest in sections of the amygdaloid end. Within subsectional areas of coronal section of the hippocampus (CA-1, CA-3, and dentate gyrus), the cyclic GMP content, the affinity of $[^3H]$QNB specific binding sites were the highest in the CA-3 and dentate gyrus regions, while the density of $[^3H]$QNB specific binding was the highest in the CA-1 area. In contrast, the fractional rate constant for the efflux of deuretiated ACh was higher in the CA-3 area than in the dentate gyrus. This study demonstrates that an enrichment of ACh content, affinity of $[^3H]$QNB binding sites and cyclic GMP content appears to occur in some specific areas of the hippocampus. These findings suggest that the dissection described in the present experiments defines areas with different degrees of cholinergic innervation. Hence such ACh dissection may be useful to study the cholinergic regulation of the cGMP content of some regions of the hippocampus.

PHS-6040
(Rev. 10-76)

Project Description:

The results of this study elucidate several points about the distribution of acetylcholine, $[^3H]$QNB binding and cyclic GMP content in rat hippocampus.

First, the acetylcholine content significantly increases from the septal end to the amygdaloid end of the hippocampus; this presumably reflects a greater density of acetylcholine terminals at the amygdaloid end. It is generally assumed that the cell bodies of hippocampal cholinergic axons are located in the medial septal nuclei. Whether the cholinergic fibers of the amygdaloid and of hippocampus come from septal nuclei is not clear at this time; this question could be examined by lesions of the fimbria. In the various coronal sections of hippocampus a high acetylcholine content is accompanied by a high cyclic GMP content. Since neither the K_d nor the Bmax for $[^3H]$QNB varies significantly in serial coronal sections from the septal to the amygdaloid end, one could infer that some of the acetylcholine terminals are innervating nicotinic receptors. The high cyclic GMP content of the amygdaloid end does not appear to be due to a concentration of muscarinic receptors higher than in other coronal section. One could speculate that this increase may be in response to the larger concentration of the endogenous agonist, acetylcholine.

A report on the distribution of acetylcholinesterase and cholineacetyltransferase in serial coronal sections of the hippocampus is not available; hence a direct comparison of the enzymes with acetylcholine content, receptor density, and cyclic GMP content cannot be made.

The CA-3 and dentate gyrus subsections are richer in cyclic GMP content and have binding sites with higher affinity for muscarinic agonists than the CA-1 subsection. Since dentate and CA-3 areas do not have a acetylcholine content higher than CA-1, the high cyclic GMP content at least, in part, could be due to a more persistent binding of endogenous acetylcholine to the muscarinic receptor which, in turn, by causing a greater stimulation of the guanylcyclase maintains a higher steady state of cyclic GMP. Our data suggest that the muscarinic receptors are heterogenous with regard to their affinity constant for the muscarinic antagonist, $[^3H]$QNB. The existence of more than one population of muscarinic receptors has been proposed. However, a better characterization of these two types of muscarinic receptors for a longer group of agonists and antagonists is in order before we assume that this suggestion has some merit. The finding that the Bmax for $[^3H]$QNB binding is higher in the CA-1 region than in the dentate gyrus confirms the results of previous studies with autoradiographic methods. While the autoradiographic studies of $[^3H]$QNB binding provide more precise information on the receptor location in a subsection, this method fails to provide a quantification of the affinity of the muscarinic receptor for the ligand, $[^3H]$QNB.

From studies fo the location of acetylcholinesterase and cholineacetyltransferase, it is difficult to deduce the distribution of muscarinic receptors. The CA-1 and CA-3 regions appear to have the largest concentration of both these enzymes. This may suggest an enrichment of cholinergic synapses, since such an inference fails to be supported either by QNB affinity, density, or by the content or turnover of acetylcholine, one must be cautious in inferring morphological considerations from the measurement of esterase or transferase activities.

In summary, the regional enrichment of acetylcholine, the higher affinity muscarinic receptor for $[^3H]$QNB, the greater density of QNB binding sites, and the high cyclic GMP content are observations of value in establishing a profile of the muscarinic receptor content and characteristics in a given brain area. While only one measurement provides limited predictive value, this cluster of parameters provide definite predictive value.

Publication:

Kinnier, W. J., Chuang, D. M., Cheney, D. L., and Costa, E.: Distribution of acetylcholine, muscarinic receptors and cyclic GMP in rat hippocampus. Neuropharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01539-02 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Different ontogenic development for soluble and membrane bound protein kinase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Kinnier	Staff Fellow	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	F. Vargas	Guest Worker	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have found an increase during development in the specific activity of cyclic AMP-dependent and calcium-dependent protein kinases of synaptic membrane from rat brain while the specific activity and cyclic AMP binding affinity of the soluble cyclic AMP dependent protein kinase was independent of the age of the animal. The concomitant rise in the membrane protein kinase with synaptic formation supports the concept that these enzymes are required for synaptic transmission.

Proposed Course:

This project has been terminated.

Publication:

Kinnier, W. J., Chikvaidze, V., Hollenbeck, R. A., Chuang, D.-M., and Costa, E.: A different development for soluble and membrane bound protein kinases of rat brain. Developmental Neuroscience 2: 154-159, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01540-02 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Newly detected biologically active enkephalin-like peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	T.D. Hexum	Guest Worker	SMRP	NIMH
Other:	I. Hanbauer	Pharmacologist		NIH
	H.-Y.T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
Section on Biochemical Pharmacology, NHL&B, NIH, Bethesda, MD

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The existence of varying quantities of enkephalin-like peptides in adrenal glands of cow, human, dog, guinea pig and rat has been demonstrated by a radioimmunoassay technique. The concentrations detected are higher in dog and cow than in guinea pig and rat. In every species, a large proportion of the total immunoreactivity is present as high molecular weight form; furthermore, these high molecular weight forms are concentrated primarily in chromaffin granules. In order to explore the physiological role of enkephalin-like peptides in adrenal, in vivo secretion of these peptides from dog adrenal was studied. Stimulation of the splanchnic nerves resulted in a voltage-dependent increase in plasma levels of the enkephalin-like peptides; their secretion was increased after injections of methacholine and morphine and these effects were blocked by atropine and naloxone, respectively. We will continue to study the physiological regulation of the secretion of these enkephalin-like peptides from the adrenal gland.

PHS-6040
(Rev. 10-76)

Project Description:

Enkephalin-like immunoreactive peptides are present in varying amounts in adrenal glands of all species studied. The chemical characterization of the enkephalin-like peptides in cow adrenal glands reveals a very high degree of heterogeneity in the molecular weights of these peptides; and furthermore, the pentapeptide, met^5-enkephalin, accounts for only a small proportion of the total immunoreactivity. Because of these observations, we have decided to study: 1) the composition and cellular location of the enkephalin-like material in adrenals of various species and 2) the secretion of these enkephalin-like peptides from dog adrenal glands where the storage is particularly abundant. The total met^5-enkephalin-like immunoreactivity in adrenal glands of various species varies over a very wide range. Much higher quantities are present in dog and cow adrenals than in the adrenals of guinea pig and rat. Rat adrenal contains only 0.05% of the enkephalin-like immunoreactivity as that present in the dog. Analysis of the enkephalin-like immunoreactivity by gel filtration chromatography followed by RIA reveals that in every species, a large proportion of the total immunoreactivity is represented by high molecular weight forms (M.W. 1000). The ratio of the high to low molecular weight immunoreactivity expressed in met^5-enkephalin is equivalent in such species to 2.6. Analysis of the various regions of bovine adrenal glands indicates that enkephalin-like material is highly concentrated in medulla but occurs also in cortex though in a much smaller quantity. Furthermore, the immunoreactive peptides appear to be concentrated primarily in the chromaffin granules since there are 2720 pmoles/mg protein in the chromaffin granules and only 52 pmoles/mg protein in the whole gland. It has been shown by immunohistochemical study that in some gland cells there is coexistence of catecholamines and enkephalin-like immunoreactivity. This observation raises the possibility that, like catecholamines, enkephalin-like peptides may be secreted into the circulation from the adrenal glands. Therefore, with the cooperation of Dr. Hanbauer, a study was initiated on the in vivo secretion of these peptides from adrenal glands. The experiment was carried out in conscious dogs with indwelling cannulae in the left lumbar adrenal vein to allow the sampling of adrenal venous blood. Plasma from the adrenal vein was found to contain a greater amount of enkephalin-like peptide (0.087 pmoles/ml) than the plasma from jugular and femoral veins or femoral artery (0.045 pmoles/ml). This finding is in keeping with the view that the adrenal gland is the source of enkephalin-like peptides present in the blood. In anesthetized dogs, the stimulation of the splanchnic nerve resulted in a rise in blood pressure and in a voltage-dependent increase in plasma met^5-enkephalin-like peptides. At 20 V, for example, stimulation always gives an increase of 150-500% over control. Biogel P2 gel filtration of plasma extracts obtained before and after splanchnic nerve stimulation revealed the presence of both high and low molecular weight enkephalin-like peptides and the latter was increased by nerve stimulation. One of the low molecular weight forms was identified to be met^5-enkephalin. Methacholine produces an effect similar to splanchnic nerve stimulation and this effect is blocked by atropine. Nicotine was found to have no effect at the doses tested (5 to 50 mol/kg). The intravenous administration of morphine produces a marked dose-dependent increase in plasma enkephalin-like peptide. This effect of the morphine was blocked by naloxone. We are currently continuing the study on the regulation of the secretion of enkephalin-like peptides from the adrenal gland.

Publications:

Yang, H.-Y.T., Hexum, T., and Costa, E.: Adrenal enkephalin-like peptides. Life Sciences, in press

Yang, H.-Y.T., Hexum, T. and Costa, E.: Opioid peptides in adrenal gland. Life Sciences, in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01541-02 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
In vivo inactivation of enkephalins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Stine	Staff Fellow	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The exact molecular mechanisms involved in the catabolism of enkephalin are still unclear, but various reports strongly suggest that Tyr-Gly-Gly may be a major metabolite produced by the action of an exopeptidase such as carboxypeptidase or dipeptidyl carboxypeptidase. In order to test which exopeptidase is involved in the catabolism of enkephalin, we have studied the effect of dipeptidyl carboxypeptidase inhibitor, captopril, on the rate of disappearance of intraventricularly injected enkephalin and on endogenous brain enkephalin content. The drugs prolongs the half-life of injected enkephalin and potentiates the analgesic effect of the injected enkephalin but fails to change the content of endogenous enkephalin in either control or stressed animals. These results suggest that dipeptidyl carboxypeptidase may not be involved in the catabolism of endogenous enkephalin. We are currently studying the effect of a carboxypeptidase A inhibitor on the enkephalin content of various brain parts of normal and stressed rats.

Proposed Course:

This project has been terminated.

Publication:

Stine, S. M., Yang, H.-Y. T., and Costa, E.: Inhibition of in situ metabolism of ^{3}H-(met^5)-enkephalin and potentiation of (met^5)-enkephalin analgesia by captopril. Brain Res. 188: 295-299, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01542-02 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Amino acid neurotransmitters and hippocampal function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Di Lauro	Guest Worker	SMRP	NIMH
Other:	R. Schmid	Visiting Fellow	SMRP	NIMH
	J. L. Meek	Phamacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neurotransmitter of the tract from the entorhinal cortex to the hippocampus (the perforant pathway) is not known, but is thought to be an excitatory amino acid, probably aspartic or glutamic acid. To learn which one is involved, analysis of putative amino acid transmitters (GABA, glycine, aspartate, glutamate and taurine) was made by high pressure liquid chromatography (HPLC) in rat hippocampus after surgical lesion of the entorhinal cortex, or lesion of intrinsic neurons in the hippocampus with kainic acid. Destruction of the perforant pathway caused a decrease in the content of aspartate while the content of the other amino acid was unchanged. The content of all the amino acids measured decreased after injection into the hippocampus of kainic acid. Lesion of the perforant pathway in kainic acid-treated rats caused a further decrease only of the aspartate content. The experiments suggest that aspartate is the neurotransmitter of the perforant pathway.

Project Description:

Identification of the neurotransmitters which are operative in various brain pathways is relatively easy when a specific histochemical localization of the transmitters or their synthetic enzymes is possible. The use of histochemical methods to locate putative amino acid transmitters (GABA, glycine, glutamate, aspartate and possibly taurine) is less successful since these compounds occur in all brain cells (glia as well as all neurons). Histochemistry will visualize these amino acids in every brain cell. Most of the evidence available to support a neurotransmitter role for amino acids has come from studies involving either iontophoresis onto cells, or measurement of high affinity uptake into slices. Unfortunately, neither of these methods can distinguish between aspartergic or glutamatergic cells or receptors. Some CNS areas receive rich amino acidergic innervation such as the substantia nigra (by GABA) and dorsal horn of the spinal cord (by glycine). This fact is reflected in a higher than average content of a particular amino acid in a selective brain area. In order to gain evidence for the existence of an aspartergic system, we have looked for a pathway that was thought to be either aspartergic or glutamatergic on electrophysiological grounds, and one which richly innervates a given brain area. For these experiments we chose the perforant pathway: a tract from the entorhinal cortex to the fascia dentata of the hippocampus.

The objectives of this study were to determine whether the perforant pathway is in fact aspartergic by measuring the amino acid content of the fascia dentata after lesion of the entorhinal cortex.

The methodology used consisted of an automated amino acid analysis system employing separation of the amino acids on a cation exchanger by high pressure liquid chromatography (HPLC) and detection by fluorescence.

Major Findings

1) The fascia dentata could be dissected free from the rest of the hippocampus when the rats were killed by focussed microwave irradiation.
2) The aspartate content of the fascia dentata decreased after a lesion of the perforant pathway; a maximal depletion is reached at 3 days after lesion. The decrease occurring in the rest of the hippocampus was much smaller.
3) There was no significant change in the content of the other amino acids measured (GABA, glutamate, taurine or glycine) after perforant pathway lesion.
4) Aspartate concentrations tended to return towards normal at 7-15 days after lesion, reflecting the known sprouting of terminals from other cells, presumably aspartergic.
5) Chemical lesion of the hippocampus with kainic acid decreased the GABA, glutamate, aspartate and taurine content of this structure.

6) Lesions of the perforant pathway in kainic acid-treated rats caused a further decrease in aspartate content over that caused by kainic acid alone.

Significance

Understanding of brain function requires knowledge of all neurotransmitter systems. Before the role of the aspartergic system can be established, major advances are needed in the mapping of these cells and the study of their receptor dynamics. Such studies demand that new tools be developed, such as specific aspartate binding methods, and isolation of receptors for antibody preparation. The demonstration that the fascia dentata has an experimentally alterable aspartergic innervation should aid in this work.

A paper describing this study is being prepared for submission to *Nature*.

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U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01543-01 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of neurotensin in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Govoni	Visiting Associate	SMRP	NIMH
Other:	J. S. Hong	Staff Fellow	SMRP	NIMH
	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to study the interaction of _neurotensin_ containing neurons with other neuronal systems, the neurotensin content of various _rat brain_ regions was studied after _haloperidol_ treatment and various _brain lesions_. Brain lesion experiments suggest that the neurotensin content of nucleus accumbens is probably located in intrinsic or short axon neurons projecting from stria terminalis. This neurotensin store increases 16 hrs after a single injection of haloperidol (2 mg/kg i.p.) and the increase was more pronounced after repeated injections of haloperidol (2 mg/kg i.p. per day for 2 weeks). The effect of haloperidol was not observed in preoptic area, hypothalamus, septum and amygdala. The specific effect of haloperidol suggests that neurotensin metabolism in nucleus accumbens may be modulated directly or indirectly through dopaminergic synapses.

PHS-6040
(Rev. 10-76)

Project Description:

Neurotensin is a tridecapeptide originally isolated from bovine hypothalamus in 1973. The physiological role of this peptide is still unclear; however, its uneven distribution in CNS, its synaptosomal location and its effect on the neurons suggest that neurotensin may participate in neuronal transmission. In order to study the interaction of neurotensin containing neurons with other neuronal systems, the distribution of neurotensin in various brain regions was studied by radioimmunoassay and then the effects of haloperidol treatments and various brain lesions on the neurotensin content of various regions were studied.

High concentrations of neurotensin were found in the preoptic area, hypothalamus, central gray of mesencephalon and certain nuclei of the limbic system i.e. septum, nucleus accumbens, amygdala. Low concentrations were observed in hippocampus, caudate-putamen and frontal cortex and none in the cerebellum.

In order to ascertain whether the neurotensin contained in nucleus accumbens was present in neurons intrinsic to this area or in neurons that project to N. accumbens from other brain areas, three lesions, such as kainic acid injection into the nucleus accumbens, hemitransection at A 4380, and cortical degeneration, were performed. The neurotensin content in nucleus accumbens was not decreased by any of the lesions, suggesting that the neurotensin contained in nucleus accumbens is probably present in intrinsic or short axon neurons projecting from bed nucleus or stria terminalis. This neurotensin store increases 16 hrs after a single injection of 2 mg/kg i.p. of haloperidol; this increase persists for 8 hrs or longer. Repeated injections of 2 mg/kg i.p. per diem of haloperidol cause a gradual and progressive increase of neurotensin. From 0.32 pmol/mg protein it increases to 0.57 pmoles/mg (1 week) to 0.62 pmol/mg (2 weeks) and reaches 0.68 pmoles/mg (3 weeks). A significant increase of neurotensin content of nucleus accumbens is obtained with 0.5 mg/kg i.p. daily for two weeks. Maximal responses are obtained with 1 mg/kg day in three weeks. The striatal neurotensin content of rats injected for two weeks with 1 mg/kg per diem of haloperidol is also increased. In these rats the neurotensin content of preoptic area hypothalamus, septum, amygdala failed to increase. The specific modulation of neurotensin content caused by haloperidol suggests a modulation of neurotensin metabolism, synthesis or utilization directly or indirectly through dopaminergic synapses.

We are currently studying the effect of various antipsychotic drugs on the neurotensin content in nucleus accumbens and striatum.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01544-01 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Histamine: a possible neuroactive substance in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Olianas	Visiting Associate	SMRP	NIMH
	S. O. Kayaalp	Visiting Scientist	SMRP	NIMH
	M. Parenti	Visiting Associate	SMRP	NIMH
	A. P. Oliver	Psychologist	SMRC	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Histamine has been proposed as a neurotransmitter in brain especially in the hippocampus. Our objective is to investigate receptor mechanisms of hippocampus. Histamine binding sites will be studied with radioactive ligands. In addition, histamine-stimulated adenylate cyclase activity will be correlated with changes of neuronal firing rates induced by histamine in the isolated hippocampal slice.

PHS-6040
(Rev. 10-76)

Project Description:

There is now substantial evidence that histamine might serve as a neurotransmitter in brain. In peripheral tissues two types of histamine receptors have been identified pharmacologically, H1 and H2 receptors. H2 receptors are coupled to an adenylate cyclase system. Our objective is to correlate the results using guinea pig hippocampus for histamine binding sites, histamine activated adenylate cyclase activity and histamine-induced changes of neuronal firing rates. These studies may reveal a potential role for histamine in brain function.

Histamine binding sites were evaluated in membrane preparations using a new H2 receptor antagonist, 2-guanidino-4-[-2-]-(2-cyano-3-methylguanidino) butyl]-thiazol (ICIAS 165).

ICIA 5165 has a higher affinity for histamine H2 receptors than most currently available drugs. Changes of spontaneous neuronal firing rates induced by histamine were evaluated in a hippocampal slice with extracellular recording electrodes. Histamine induced changes of adenylate cyclase activity was studied in homogenates of guinea pig hippocampus. We observed that ICIA 5165 binds to sites in guinea pig brain that have characteristics of the H2 receptors; for example, brain areas with reported histamine activated adenylate cyclase activity had the highest affinity for this ligand. In addition, drugs that interact with H2 receptors in peripheral tissues displaced the ligand from brain membranes. Histamine stimulated adenylate cyclase of hippocampus resulted in a half maximal activation of about 5 µM. When histamine was applied to a hippocampal slice, firing of the neurons increased. Interestingly, the half maximal firing rate for histamine was about 5 µM, the same concentration found for half maximal activation of adenylate cyclase activity.

Presently, little is known about the role of histamine in brain function. There is evidence however that tricyclic antidepressants may inhibit H2 receptor sites. Our studies are the first to correlate biochemical responses to histamine with physiological responses in brain. These studies may provide the needed information to establish a role for histamine in normal brain function.

Future studies will be directed towards providing a more critical biochemical and electrophysiological approach to histamine in hippocampal function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
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NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01545-01 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Enkephalin-like immunoreactivity in chromaffin cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Stine	Staff Fellow	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Enkephalin-like immunoreactive material present in isolated bovine chromaffin cells is released spontaneously when cells are incubated in Krebs-Ringer buffer. This release is increased by potassium and by acetylcholine stimulation. In both cases the stimulated increase in release is calcium-dependent. Preliminary evidence indicates that under these experimental conditions the response to acetylcholine appears to be mediated by nicotinic receptors. Biochemical characterization indicates that enkephalin-like material is contained in and released from the chromaffin cells as high molecular weight forms.

PHS-6040
(Rev. 10-76)

Project Description:

Enkephalin-like immunoreactivity has been detected in adrenal medullary cells of all species studied. It is found in particularly high amounts in dog and bovine adrenal glands. Chemical characterization of the immunoreactive material in bovine adrenal medulla has revealed that enkephalin-like peptides in medullary cells are stored predominantly as high molecular weight forms in chromaffin granules. The physiological role(s) of this enkephalin-like immunoreactivity in chromaffin granules is not clear, though a hormonal role has been suggested. In order to explore this hypothesis we have decided to study whether the enkephalin-like immunoreactivity in chromaffin cells can be released by physiological stimuli.

Chromaffin cells isolated from bovine adrenal glands were found to contain mainly high molecular weight forms of enkephalin-like immunoreactive peptides. These enkephalin-like peptides are released spontaneously when cells are incubated in Krebs-Ringer solution. This release was increased by potassium and by acetylcholine stimulation. In both cases this stimulation was found to be calcium-dependent. Preliminary evidence suggests that under the conditions employed in these experiments the acetylcholine-induced release appears to be mediated by nicotinic receptors because the release is affected also by nicotine and not by metacholine. In addition, acetylcholine-stimulated release is completely blocked by hexamethonium but not by atropine. The enkephalin-like immunoreactive peptides released by acetylcholine stimulation were found to consist mainly of high molecular weight forms.

We are currently investigating the possible role of opiate-like material (located in afferent axons) on the release of high molecular weight enkephalin-like material from chromaffin cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01546-01 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neurotransmitters and neuromodulators: A study of their interaction on receptors of neuroblastoma cell lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Gwynn	Guest Worker	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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SECTION
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The clonal neuroblastoma cell line, N_4TG_1, was used to elucidate the interaction of opiate receptors with muscarinic, benzodiazapine and tricyclic antidepressant receptors. It was found that chronic treatment (14 days) of N_4TG_1 cells with opiate receptor agonists increases their binding capacity for muscarinic agonists without changing the binding capacity for opiate agonist. The mechanism of this interaction is being investigated.

Project Description:

In spite of the considerable progress made in the characterization of the opioid receptor per se, very little is known about its molecular and functional characteristics. Since it appears quite clear that modulation of opioid receptor number or characteristics is not responsible for the development of tolerance to narcotic drugs, the biochemical changes might be one step removed from agonist receptor interactions. A number of studies conducted in the central and peripheral nervous system have shown that acute and chronic opiate treatment can modulate the number of receptors available for the neurotransmitters. To overcome difficulties due to cellular heterogeneity in neural tissue, we have chosen to utilize a simple cellular system, the clonal neurotumor cell line N_4TG_1, to elucidate the biochemical sequelae triggered by the activation of opiate receptors.

We have first characterized the receptor populations of the model N_4TG_1 system. Studies with intact cells and crude membrane preparations revealed high-affinity binding sites for the agonist ^{3}H-etorphine and the kappa agonist, ^{3}H-ethylketazocine, which were displaceable by the specific narcotic antagonist naltrexone. The muscarinic antagonist ^{3}H-quinuclidiayl benzylate (^{3}H-QNB) labeled both high and low affinity sites, both displaceable by atropine and oxotremorine. In N_4TG_1 similar to other brain cells, the agonist binding to both opioid and muscarinic cholinergic receptors is regulated by the nucleotide analogue Gpp(NH)p. A considerable number of benzodiazepine and tricyclic antidepressant high affinity binding sites, but not β-adrenergic binding sites, were found in this neuroblastoma cell line.

In order to determine the effects of chronic narcotic treatment on these receptor populations, cells were grown in the presence of increasing doses of morphine sulfate (MRP) or equimolar amounts of Na_2SO_4 for a period of 14 days. The in vivo binding of the muscarinic antagonist ^{3}H-QNB was significantly enhanced in the cells treated chronically with MRP while the bindings of ^{3}H-etorphine and ^{3}H-diazepam were identical to that of the control cells.

Guanylate cyclase, a Ca^{++}-dependent enzyme, is regulated by the muscarinic cholinergic receptor in many tissues. Our preliminary investigations showed that MRP can regulate the steady-state level of calcium in N_4TG_1. The flux of ^{45}Ca in intact N_4TG_1 cells was inhibited by acute MRP treatment and significantly enhanced by chronic MRP treatment. Furthermore, the increase in cGMP induced by carbamylcholine in these cells was antagonized by the opiate agonist etorphine.

Chronic MRP treatment of N_4TG_1 has been shown by others (Dawson et al., 1980) to alter the complex carbohydrate composition of the cell surface. Since negatively charged glycoproteins and glycolipids may constitute membrane receptors and ionic channels, it is plausible that these changes in membrane composition may underlie the effects of opiate agonists on the functioning of the muscarinic cholinergic receptor in N_4TG_1.

Clonal cell lines of neuroblastoma and glioma cells have proved to be a suitable model system for studying molecular mechanisms in the organization of several neurotransmitter receptors. Now we intend to use this model to study how cotransmitter substances (i.e., endogenous opiates, substance P, etc.) function as "enablers" of the receptor function for primary transmitters.

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PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Fate of enkephalin-like peptide released from adrenal gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Harsing	Visiting Associate	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several lines of evidence indicate that enkephalin-like peptides in adrenal gland can be released into circulation. In order to explore the role of these released peptides, the stability of the enkephalin-like peptides extracted from adrenal glands in plasma was studied. The high molecular weight enkephalin-like peptides were found to be very stable, while, the low molecular weight enkephalin-like peptides were rapidly degraded in plasma. Our preliminary result also suggests that there may be an enkephalin binding peptide or protein in plasma. Hence it is possible that enkephalin may be released into circulation in some kind of protected form such as bound to a large peptide or protein. In fact presence of enkephalin in blood has been documented in spite of its very short half-life. We are planning to explore the possible target tissues for both high and low molecular weight forms of enkephalin-like peptides in periphery.

Project Description:

There is ample evidence suggesting that enkephalin-like immunoreactive peptides in adrenal glands can be released into blood; however, the fate of these released peptides is not clear at present. In order to explore the role of these enkephalin-like peptides, secreted into circulation from adrenal glands, we have decided to study 1) the stability of the enkephalin-like peptides of adrenal gland origin in plasma and 2) the possible peripheral target tissue(s) for the adrenal medullary enkephalin-like peptides.

The high molecular weight enkephalin-like peptides were found to be very stable in plasma, while met^5-enkephalin and other low molecular weight enkephalin-like peptides were very rapidly degraded in plasma. In spite of this observation, the existence of met^5-enkephalin in blood has been well documented. These seemingly irreconcilable observations suggest that met^5-enkephalin may be released into blood in some kind of protected form from adrenal glands or that there is a continuous supply of met^5-enkephalin in blood. Other preliminary experiments seem to indicate that there may be enkephalin binding peptide or protein in plasma. We are planning to 1) further characterize this enkephalin binding protein in plasma and 2) search for peripheral target tissues for adrenal medullary enkephalin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01548-01 SMRP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Afferent control of serotonergic pathways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. M. Forchetti	Visiting Fellow	SMRP	NIMH
Other:	J. L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The transmitters released by afferents to the serotonin (5HT) neurons of the median raphe nucleus were examined. We injected into that nucleus agonists and antagonists of the neurotransmitter GABA, and then measured 5HT turnover and/or 5-hydroxyindoleacetic acid content in the hippocampus. Turnover of 5HT was estimated with the aid of high pressure liquid chromatography (HPLC) by measuring the probenecid-induced accumulation of 5-hydroyindoleacetic acid. We found that intra raphe injection of the GABA agonist muscimol decreased hippocampal 5HT turnover. Conversely, the GABA antagonists, picrotoxin and bicuculline increased GABA turnover. The data show that raphe contains GABA receptors; the stimulation of these receptors has a tonic inhibitory control over the firing of raphe 5HT neurons.

Project Description:

Pharmacological research into the role of serotonin in brain function has for many years involved oral or intraperitoneal administration of drugs followed by measurement of 5HT turnover in brain. Because of the integrated nature of the brain, such experiments provide information on direct and indirect drug actions on 5HT receptors. Hence the results represent the net effect of all actions of the drug on all neurons which influence 5HT cells. It is important to gain more information on the axons afferent to 5HT neurons because by a pharmacological regulation of those afferents one can perhaps manipulate sleep, emotion, eating and sex, which are the major areas of human behavior in which 5HT is operative. For example, it is thought that part of the antianxiety effect of benzodiazepines might be due to a potentiation of the GABAergic regulation operating on 5HT neurons. For a better understanding of the transmitters operate on the afferents to 5HT neurons and how drugs might affect 5HT function, we have injected neurotransmitter agonists and antagonists directly into an area containing the 5HT cell bodies (the raphe nuclei of the midbrain) and then measured the turnover of 5HT in an area innervated by that nucleus.

The objective of this study was to determine pharmacologically whether the neurotransmitter GABA modulates the activity of 5HT cells, directly.

The methodology employed involved the injection of GABA agonists and antagonists into the median raphe nucleus of rats, and the measurement of 5HT turnover in the hippocampus. Changes in 5HT turnover were estimated by measuring the rate of 5HIAA (5-hydroxyindoleacetic acid) accumulation after blocking its efflux with probenecid, or the spontaneous increase in the steady state level of 5HIAA. The 5HIAA was measured by high pressure liquid chromatography (HPLC).

Major Findings

1) Injection of muscimol (a GABA agonist) decreased the steady state content of 5HIAA, and decreased the accumulation of 5HIAA after probenecid. This decrease in 5HT turnover indicates that GABA inhibits 5HT cell firing.
2) Injection of picrotoxin or bicuculline (GABA antagonists) increases the steady state content of 5HIAA. These findings indicate that 5HT turnover is increased and suggest that these drugs remove a tonic inhibitory action of GABA on 5HT cells.
3) Muscimol can reverse the effect of picrotoxin.
4) The injection of diazepam (a minor tranquilizer) potentiates this effect of muscimol indicating that diazepam can in fact facilitate the tonic inhibitor of 5HT cells by GABA.

The findings help to fill in a major gap in our knowledge of the serotonergic system; we know where 5HT fibers go, but we knew very little about how 5HT neurons are regulated, transsynaptically. This study, and future work with local injection of neurotransmitters or drugs that modify neurotransmitter function, directly into the areas containing 5HT cell bodies, will reveal much more detailed information about control of 5HT activity than has previously been available. Together with existing knowledge on the effects of peripheral administration of drugs, a new understanding of how mental disorders might be related to the function of 5HT neurons will be developed, along with the possibility of new methods of treatment for these malfunctions.

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PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Down regulation of imipramine binding sites in rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Kinnier	Staff Fellow	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In rats receiving repeated injections of imipramine, there is a reduction in the number of high affinity binding sites for [^{3}H]imipramine and [^{3}H]dihydroalprenolol present in crude synaptic membrane preparations from various brain structures. The location of the sites that become subsensitive to the two ligands did not coincide; the binding sites to [^{3}H]imipramine became subsensitive in the hippocampus but not in cortex or cerebellum. In contrast the binding sites to [^{3}H]dihydroalprenolol became subsensitive in cortex and cerebellum but not in hippocampus. It can be suggested that in rats repeatedly treated with imipramine the down regulation of β-adrenergic receptors does not depend on the down regulation of the high affinity binding sites for imipramine. Such a dissociation is supported further by experiments with rats treated with iprindol.

PHS-6040
(Rev. 10-76)

Project Description:

These studies demonstrate that repeated injections of imipramine (10 mg/kg, i.p.) for 10 days can reduce the number of [^{3}H]imipramine binding sites (Bmax) in the membrane prepared from hippocampus, suggesting that this high affinity binding to innervated receptor can be similarly down regulated. It seems unlikely that such a down regulation is an artifact due to a persistent occupancy of the imipramine binding sites by this agonist. The membrane preparations used in this study had been washed extensively to remove the residual imipramine bound to the membrane sites; this treatment should have been effective in eliminating the imipramine bound to the membrane even though the affinity of [^{3}H]imipramine binding was increased as a result of chronic imipramine treatments. The reduction in the number of the imipramine binding sites is a time-dependent process; a single injection fails to reduce the number of imipramine binding sites. Apparently, the modification of [^{3}H]imipramine binding elicited by repeated injections of imipramine is not a uniform response of the various populations of the imipramine recognition sites present in various brain structures. In fact, under our experimental conditions, down regulation of imipramine binding fails to occur in the membranes prepared from cerebellum and cortex of rats receiving daily imipramine injections for ten to fourteen days. The reason for this difference can not be explained by our experiments. As a working hypothesis, one can propose that the binding sites for imipramine are regulated differently in various brain areas. Our studies could suggest that when the number of binding sites for imipramine is decreased by chronic imipramine, a concomitant reduction in the number of ^{3}H dihydroalprenolol binding sites fails to occur. However, reports by Raisman et al. (1980) have shown that in cortex after daily injections of imipramine, lasting longer than in our experiments, both the [^{3}H]imipramine and [^{3}H]dihydroalprenolol binding sites are down regulated. Chronic treatment of rats with iprindol reduce the number of β-receptors but fail to change the number or affinity of imipramine binding sites, suggesting that the action of iprindol on β-receptor desensitization is mediated by unidentified binding sites, specific for iprindol. One can not, however, exclude the possibility that the sites that recognize imipramine or iprindol are also specific for an endogenous regulator (peptide?) which modulates β-receptor function.

The reason why the number of imipramine binding sites in hippocampus decreases before that of other brain areas is unclear; if the higher affinity binding denotes binding to synaptic sites, it seems logical to speculate that the imipramine recognition sites in hippocampus are located in conjunction with synapses different from those of cortex and cerebellum. Alternatively, cortical and cerebellar sites for [^{3}H]imipramine binding may be presynaptic and regulate the reuptake of a catecholamine transmitter acting on β-receptors. All these possibilities are now under investigation.

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